Orangutan Genes in Space and Time:

The Impact of Evolutionary Processes of Diversification on Bornean Orangutans

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Abstract

Underlying patterns of genetic diversity and differentiation in species, and ultimately responsible for the staggering diversity of life forms on earth, are processes of evolutionary change that are not well understood. In this thesis I therefore delved into the environmental and biological mechanisms that shape patterns of gene flow and structure genetic diversity in orangutans. In particular, I investigated the effects of the Pleistocene glaciations, volcanic eruptions, and riverine barriers, as well as the effects of sex-biased dispersal. I focused on the orangutans of Borneo, as this Southeast Asian island is conspicuous for its high biotic diversity and endemism.

I examined the factors that have shaped historical patterns of gene flow. In order to achieve this, I was able to capitalize on a dataset that encompassed the largest number of Bornean orangutan populations, as well as the largest genetic dataset for this species to date. My results provided evidence for a complex Bornean evolutionary history, with a probable confinement of Bornean orangutans in a Pleistocene refugium, which was followed by a recent expansion throughout the island. Furthermore, population differentiation was indicative of historical female philopatry and male-mediated gene flow, with rivers acting as natural barriers to dispersal. Zooming in on the spatio-temporal scale, I investigated current patterns at a study site at which detailed behavioral, spatial and genetic information was available. My results support an extreme pattern of female philopatry with strong male-biased dispersal. Moreover, a detailed pedigree reconstruction using different DNA marker systems provided conclusive evidence that females form spatially stacked matrilineal clusters. In addition, my findings hinted at important issues affecting the power to disentangle relatedness patterns in non-gregarious species such as orangutans. These became especially prominent in my subsequent meta-analysis, spanning various Bornean populations, as well as one Sumatran. Here, I provided insights into the biological and methodological issues that may lead to discrepancies between inferences drawn from genetic and behavioral data. In non-gregarious species, results will be misleading when averaged pairwise relatedness estimates are used to determine sex-biased dispersal, without taking detailed spatial and behavioral information into account. Nonetheless, through the integration of genetic and behavioral data, I was able to show that the model of female philopatry and male-biased dispersal holds for both orangutan species.

Zusammenfassung

Die Muster und Prozesse, welche zur genetischen Differenzierung und Artbildung, und damit verbunden auch zur atemberaubenden Diversität der Lebensformen auf der Erde führen, sind immer noch nicht vollumfänglich verstanden. In meiner Doktorarbeit befasste ich mich daher eingehend mit jenen ökologischen und biologischen Mechanismen, welche die genetische Diversität von sowie den Genfluss zwischen Orangutan- (Pongo spp.) Populationen beeinflussen. Dabei untersuchte ich nicht nur jene Einflüsse, welche durch Eiszeiten im Pleistozän, Vulkanausbrüche und Flüsse als natürliche Barrieren für Genfluss hervorgerufen wurden, sondern auch solche, die durch geschlechtsspezifische Abwanderungstendenzen verursacht wurden.

Der Fokus meiner Arbeit lag auf mehreren Orangutanpopulationen in Borneo, da diese südostasiatische Insel ein hervorragendes Beispiel für hohe Biodiversität sowie das häufige Vorkommen endemischer Arten darstellt. Um die Faktoren zu ergründen, welche die historischen Genflussmuster geformt haben, erarbeitete ich einen Datensatz, der die bis anhin grösste Anzahl von Orangutanpopulationen in Borneo sowie die meisten genetischer Proben dieser Art umfasst. Meine Resultate lassen auf eine komplexe evolutionäre Geschichte der Orangutans auf Borneo schliessen, welche höchstwahrscheinlich durch klimatische Veränderungen im Pleistozän in ein Refugium zurückgedrängt wurden und von diesem sie anschliessend wieder ganz Borneo besiedelt haben. Die Muster der genetischen Differenzierung zwischen den einzelnen Populationen deuten zudem auf seit langem bestehende weibliche Philopatrie und durch Männchen verursachten Genfluss hin. Dabei stellen Flüsse starke natürliche Barrieren für Migration dar. Um den geschlechtsspezifischen Genfluss im Detail zu analysieren, untersuchte ich exemplarisch eine Population, für welche nebst genetischen Daten auch detaillierte Informationen über das Verhalten und die Bewegungsmuster der einzelnen Orangutans verfügbar waren. Meine Resultate bestätigen, dass die bereits im historischen Kontext aufgezeigten geschlechtsspezifischen Migrationsmuster auch kontemporär vorhanden sind. Darüber hinaus zeigte eine detaillierte Stammbaumrekonstruktion, basierend auf verschiedenen DNS-Markersystemen, deutlich, dass sich die Territorien weiblicher Orangutans derselben Matrilinie räumlich stark überlagern. Zudem weisen meine Ergebnisse auf ein wichtiges methodologisches Problem hin, welches die statistische Aussagekraft beim Aufschlüsseln von Verwandtschaftsverhältnissen in nicht in Gruppen lebenden Arten, wie Orangutans, betrifft. Diese Probleme traten insbesondere zum Vorschein, als ich in einer Meta-Analyse sämtliche Populationen in meinem Datensatz auf kontemporäre Genfluss- und Verwandtschaftsmuster analysierte. Basierend auf diesen Erkenntnissen diskutiere ich detailliert die methodologischen und biologischen Probleme, welche zu Diskrepanzen zwischen Schlussfolgerungen aus genetischen und Verhaltensdaten führen können. Die Methode, geschlechtsspezifische Migrationsmuster durch paarweise Vergleiche von Verwandtschaftsgraden von Männchen und Weibchen zu ermitteln, funktioniert bei Arten, die nicht in Gruppen leben, nicht ohne die Berücksichtigung von räumlichen Verhaltensdaten. Erst durch das Kombinieren von genetischen und Verhaltensdaten war ich daher in der Lage aufzuzeigen, dass das Modell von weiblicher Philopatrie und männlich-spezifischem Genfluss auf beide Orangutan Arten zutrifft.

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1 Introduction

1.1 Unraveling the evolutionary processes of diversification

The staggering diversity of life forms on earth has propelled the field of evolutionary biology, yet the processes driving evolutionary change, central to this field, are not well understood and often debated (Coyne & Orr 2004b). The initial contention surrounding the definition of life forms or species has been replaced by a widespread acceptance of the popular biological species concept, which involves reproductive isolating mechanisms (Mayr 1942; Coyne & Orr 2004b). From a genetic standpoint, speciation occurs when the genetic diversity in populations reaches fixation, and there is no gene flow among populations to homogenize the different gene pools (Dobzhansky 1951). In this course of events, the structuring of genetic diversity among populations plays a critical role. Driving this structuring are various evolutionary processes operating at different levels, such as environmental and biological mechanisms (Dobzhansky 1951). Large-scale environmental factors such as occasional climatic fluctuations can open up new channels to gene flow or impede it, thus profoundly influencing the spatial distribution of genetic diversity. At the same time, biological factors characteristic of species, including generation time and breeding structure, determine the effects of the local processes of migration, selection and drift in producing patterns of genetic diversity and differentiation (Sugg *et al.* 1996; Storz 1999). But how these environmental and biological factors interact to shape genetic diversity and ultimately lead to speciation is still an area of research requiring clarification.

Numerous large-scale environmental processes such as landmass reconfigurations and climatic fluctuations have been invoked to explain diversification: these may change the landscape, producing heterogeneous habitats or topographical discontinuities that have important effects on gene flow. To take just one example, the long isolation of the tropical island of Madagascar from other landmasses, as well as its distinct bioclimatic zones, are deemed responsible for the staggering diversity of lineages unique to this island (Vences *et al.* 2009). More recently, the climatic changes of the Pleistocene forced many different species across the globe into isolated refugia in which divergence, and at times speciation, took place (Hewitt 2004; Provan & Bennett 2008; Hofreiter & Stewart 2009).

Biological factors too have an important role in determining patterns of genetic diversity and differentiation. These include life history traits such as generation time and social organization, as well as habitat requirements and sensitivity to geographical barriers (Avise 1998; Bohonak 1999). Such biological factors shape regular local evolutionary processes such as migration between populations, and local adaptation and drift, and lead to species-specific responses to environmental changes. Thus, co-distributed species that share a common history sometimes differ in their phylogeography, or spatial distribution of gene lineages (Avise 1992; Lamb *et al.* 1992; McGovern *et al.* 2010). Particularly the social organization and breeding structure of a species can have a profound impact on the response to a particular historical event. In animal species, where sex-biased dispersal is common, patterns of gene flow for females and males differ, resulting in a sex-specific apportionment of genetic diversity that affects the maintenance and loss of genetic diversity (Avise *et al.* 1987; Storz 1999). Such is the importance of sex-biased dispersal

that it was proposed to lead to fast speciation by augmenting subdivision and promoting the fixation of chromosomal rearrangements (Wilson *et al.* 1975; Bush *et al.* 1977). This mode of speciation is now contested for some mammalian species, but it is in any case recognized that the subdivision generated by sex-specific gene flow can increase the probability of rapid drift-mediated speciation in a situation with geographical or ecological isolation (Melnick *et al.* 1984; Storz 1999).

Both environmental and biological factors produce the evolutionary dynamics responsible for diversification, but their relative impacts and their interaction are poorly comprehended (Coyne & Orr 2004b; Vences *et al.* 2009). One of the most exciting geographical regions in which to examine the impact of these processes on species diversification is the region of Sundaland: the shallow continental shelf in Southeast Asia including the Malaysian peninsula, and the islands of Borneo, Sumatra, Java and Palawan (Bird *et al.* 2005). Located in the tropics, this hotspot of taxon diversity and endemism is a remarkable natural laboratory for such an investigation. Sundaland was subject to dramatic environmental processes such as volcanic eruptions and repeated episodes of isolation and connection of the islands as a result of sea level fluctuations during the interglacial and glacial periods of the Pleistocene. Additionally, the region is rich in topographical features such as mountain chains, rivers and lakes. These characteristics suggest a link between the region's dynamic environmental forces and high species diversity.

Among the most enigmatic species found in Sundaland are the orangutans (*Pongo* spp.), highly endangered taxa and the only Asian great apes. Formerly widespread throughout Southeast Asia, orangutans are currently restricted to the islands of Sumatra and Borneo, as two distinct endemic species. Orangutans constitute exciting species to examine the processes of evolutionary diversification, in particular, the extent to which the major environmental changes in Sundaland and the characteristic biological traits of orangutans have produced current phylogeographic patterns. The features that make orangutans particularly interesting include their extremely slow life histories with long generation times (Delgado & Van Schaik 2000; Wich *et al.* 2009a), which imply high susceptibility to drastic environmental changes. As rainforest canopy-bound species, the strict habitat requirements of orangutans should render them extremely sensitive to discontinuities in the forests, posing important barriers to dispersal. These biological characteristics, that is, their slow life histories and habitat restrictions, combined with small population sizes, are attributes shared among species with high extinction risks (Purvis *et al.* 2000), making it all the more alluring to study. Furthermore, there are behavioral indications for extreme male-biased dispersal in orangutans (Galdikas 1985b; van Schaik & van Hooff 1996; Singleton & van Schaik 2002), which is expected to have an impact on orangutan population genetic structure. Nevertheless, this dispersal pattern is not confirmed by genetic studies of contemporary gene flow, as these produce conflicting evidence (Utami *et al.* 2002; Goossens *et al.* 2006b; Morrogh-Bernard *et al.* 2010), and hence the issue is unclear. In addition to all these attributes, orangutans are highly charismatic species which constitute the most basal clade in the phylogeny of the extant great apes. Hence, resolving their biological characteristics is also of use for comparative studies of the great apes and as a way to reconstruct ancestral traits.

Overall, orangutans present us with a fascinating opportunity to examine the coupled effects of extrinsic environmental processes and intrinsic biological processes in allowing or impeding gene flow, with consequences for reproductive isolation and speciation. How

did the Pleistocene climatic fluctuations, volcanic eruptions and landscape features of Sundaland affect patterns of gene flow and structure genetic diversity in the orangutans? Is there a signature from historical sex-biased dispersal in their population structure? Do contemporary patterns of dispersal and gene flow match historical patterns? I aim to answer these questions through the use of extensive sampling, molecular techniques, and the integration of data from field studies. In the sections that follow, I briefly describe orangutan biology, before reviewing what is known so far on the historical and contemporary patterns of dispersal and gene flow. I then outline my specific aims, the molecular tools available, and provide a structural guideline on the thesis chapters.

1.2 Orangutans in a nutshell

Orangutans stand out among the other great apes and mammals as a result of several characteristic and prominent features. Not only are they the only Asian great apes, but also the largest extant arboreal mammals, with specialized anatomical adaptations for an arboreal lifestyle (Delgado & Van Schaik 2000; Oishi *et al.* 2008). Accordingly, they do not show the distinctive knuckle-walking found in chimpanzees and gorillas (Delgado & Van Schaik 2000; Oishi *et al.* 2008). Orangutans inhabit rainforests that range from dipterocarp lowland and hill forests, to freshwater swamp and peat swamp forests, generally in close proximity to water in the form of rivers or swamps. However, orangutans have also been found in poorer areas including heath, mangrove forests and even submontane forests, often adjacent to richer habitats (Morrogh-Bernard *et al.* 2003; Husson *et al.* 2008). Possibly as a result of their arboreality, they have a slower life history compared to the other great apes - with the exception of humans - with later ages at first reproduction, longer inter-birth intervals and longer generation times (Wich *et al.* 2009a).

As in a variety of other mammals, males and females show dimorphism in size and appearance. But a striking feature singles out orangutans among apes: the unusual male bimaturism involving the presence of two co-existing male morphs, possibly pursuing different reproductive strategies. While flanged males are characterized by fully developed secondary sexual characteristics including larger body size, large cheek pouches termed flanges, and a fully developed throat sac (Utami *et al.* 2002; King *et al.* 2011), unflanged males are recognized by the absence of these features.

In contrast to most other diurnal primates, orangutans do not form discrete social units, and are thus deemed semi-solitary or non-gregarious (Galdikas 1985a; Rodman & Mitani 1987; van Schaik 1999; Singleton & van Schaik 2002). Nevertheless, and although orangutans spend most of their time alone or with dependant offspring, they are sometimes observed in female-male consortships (mate-guarding associations), or aggregating in parties at fruit trees or as travel bands (Rodman & Mitani 1987; van Schaik & van Hooff 1996; van Schaik 1999). Furthermore, despite low inter-individual association, there is evidence for preferential association that could be underpinned by female kin structures (Singleton & van Schaik 2002), but this has as of yet not been confirmed through genetic studies of relatedness.

The low levels of sociality observed in the orangutans, however, do not preclude exceptionally high intelligence and innovation abilities (Deaner *et al.* 2006; van Schaik *et al.* 2006). Furthermore, they display a rich material culture (van Schaik *et al.* 2003).

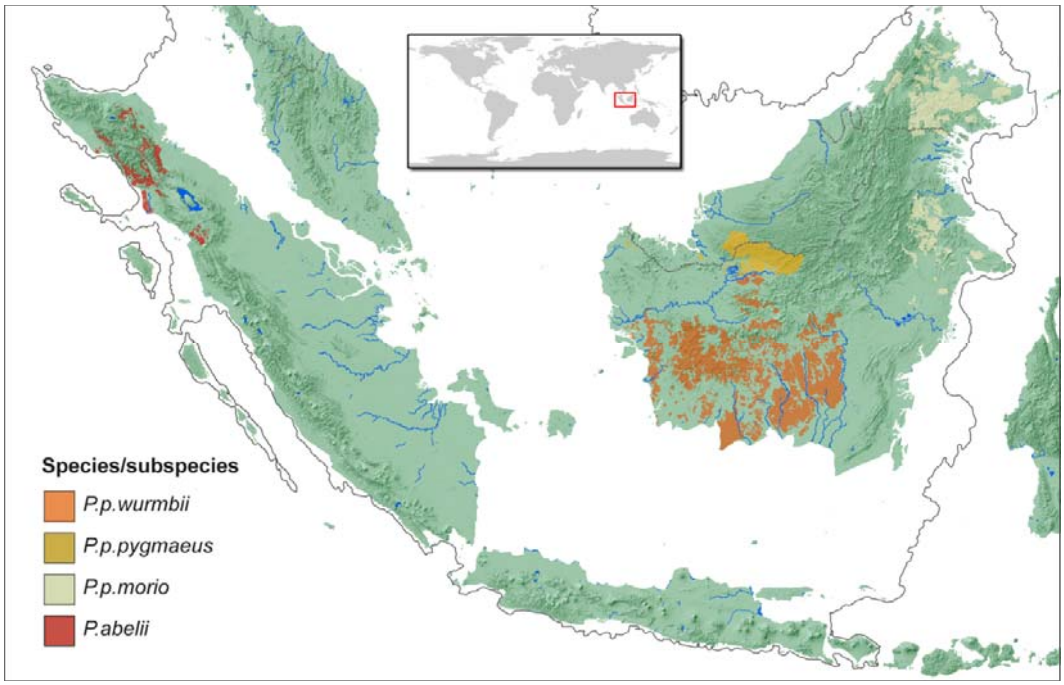
While field observations have contributed vastly to the behavioral and ecological study of orangutans, genetic studies of orangutans lag behind, with the conspicuous absence of a solid and detailed phylogeographic and population genetic characterization. Such a characterization does not only provide us with a framework within which to understand morphological and behavioral variation in orangutans, including culture, but also, and more central to this study, it allows us to examine what gave rise to current patterns of genetic diversity.

1.3 Orangutans in space and time: an unresolved framework

Current species distribution

Orangutans are found on the islands of Borneo and Sumatra as two distinct species, *Pongo pygmaeus* and *Pongo abelii*, respectively (IUCN 2010) (Fig. 1.1). Today, it is estimated that approximately 50,000 Bornean and 6,000 Sumatran orangutans survive (Wich *et al.* 2008). Within Borneo, three subspecies are recognized based on morphological differences: *P. pygmaeus wurmbii*, *P. pygmaeus pygmaeus* and *P. pygmaeus morio* (Groves 2001).

Fig. 1.1 Current distribution and taxonomic classification of orangutans in Sundaland. Adapted from E.P. Willems.



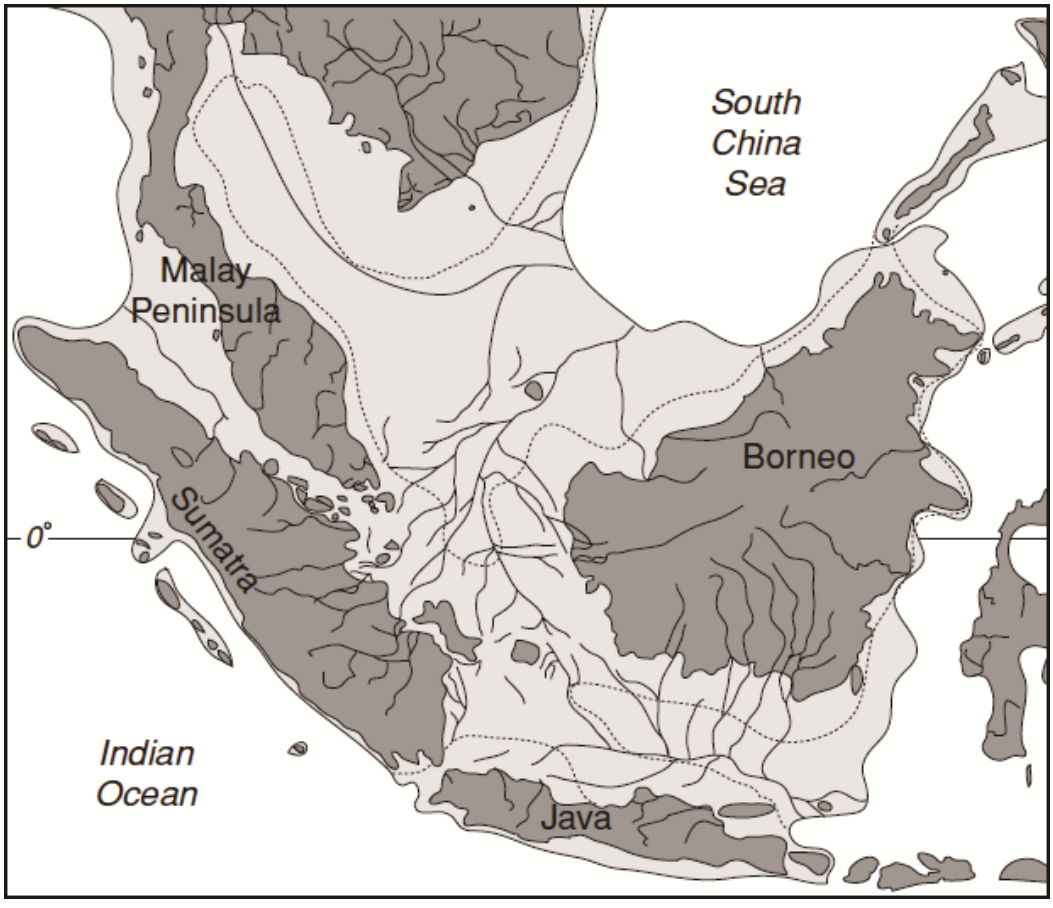
The current patchy distribution of orangutans, limited to Borneo and northern Sumatra, contrasts with that of their ancestors, who ranged expansively throughout Southeast Asia until the end of the Pleistocene. Indeed, *Pongo* fossils from that epoch have been recovered from sites in southern China, Vietnam, Laos and Java, as well as Borneo and Sumatra (Hooijer 1948; Kahlke 1972; Bacon & The Long 2001; Earl of Cranbrook 2010). But what led to the current distribution as well as population structure of orangutans is as yet an open question.

1.4 The effects of the environment

The puzzling inter-island differentiation

It is hypothesized that orangutans originated on the Southeast Asian continental mainland, and dispersed from there, reaching Sumatra first and thereafter Borneo and Java (Harrison *et al.* 2006). These movements were restricted by the presence of an extensive network of paleo-rivers that dissected the region of Sundaland (Fig. 1.2), which comprises a shallow continental shelf encompassing peninsular Malaysia, Borneo, Sumatra, Java and other islands (Bird *et al.* 2005; Harrison *et al.* 2006). Sundaland has been subject to dramatic geological and environmental forces over the past 2 million years, including marked landmass configuration rearrangements, successive climatic oscillations and massive volcanic eruptions (Rampino & Self 1992; Verstappen 1997; Hall 2002), all of which have affected the movements and survival not only of orangutans but also of other species. Dispersal from one island to another could occur during the periodic cold glacial periods of the Plio-Pleistocene, when decreasing sea levels, dropping by as much as 120 m below present levels, exposed the continental shelf between islands (Voris 2000; Harrison *et al.* 2006). By contrast, during the recurrent interglacial periods, sea levels rose and flooded the continental shelf, resulting in a separation between islands (Voris 2000; Bird *et al.* 2005). Borneo and Sumatra have now been disconnected from each other since at least the end of the last glacial period around 10 ka, yielding a minimum time since gene flow between Bornean and Sumatran orangutans became impossible.

Fig. 1.2 The paleo-rivers geographical barriers to historical dispersal of orangutans in Sundaland. Adapted from Harrison et al. (2006).



While the last glacial period ended relatively recently, Bornean and Sumatran orangutans show notable behavioral and morphological differences, although considerable intra-island variation exists (MacKinnon 1973; Courtenay *et al.* 1988; Delgado & Van Schaik 2000). Separation between the islands is also strongly mirrored in the molecular distinction that started to become evident in the 80's and 90's following a series of biochemical, karyotypic and genetic studies (Bruce & Ayala 1979; Seuánez 1982; Janczewski *et al.* 1990; Ryder & Chemnick 1993; Xu & Arnason 1996). Among these findings were the island-specific inversion patterns of two chromosomes as well as high differentiation at the level of the mitochondrial DNA (mtDNA) (Ryder & Chemnick 1993; Schempp *et al.* 1993b; Xu & Arnason 1996), which hinted that gene flow might have been halted earlier than expected. The chromosomal inversion patterns are particularly interesting given their potential involvement in drift or adaptation mediated speciation (Bush *et al.* 1977; Kirkpatrick & Barton 2006). One caveat of these early molecular studies however was the usage of very small sample sizes comprising captive individuals whose origins could not be fully attested. Nevertheless, the high genetic differentiation of Bornean and Sumatran orangutans is congruent with recent evidence for reduced hybrid fitness in captive orangutans (Cocks 2007). As a result of these findings, Bornean and Sumatran orangutans have each been elevated to the species status (IUCN 2010), a decision not entirely uncontested (Muir *et al.* 1998; Fischer *et al.* 2006).

Debated population histories

The enigmatic high inter-island differentiation of orangutans despite recurrent Pleistocene connections and potential opportunities for dispersal between the islands prompted further molecular studies of orangutan phylogeography and the colonization patterns that gave rise to it. Demographic histories to explain current population genetic structure were put forth.

In an early study, Muir (2000) proposed a scenario in which Bornean populations had remained stable while Sumatran populations had undergone local extinctions, possibly due to the volcanic super-eruption of Mount Toba around 74 ka, considered to be the largest of the Quaternary (Ninkovich *et al.* 1978). Based on the greater extent of mitochondrial genetic diversity within Sumatra compared to Borneo, as well as shared haplotypes between the islands, Muir (2000) suggested that Sumatran diversity was the result of recolonization from Borneo and other islands. Here too, however, the uncertain provenance of the rehabilitant and zoo orangutans left the issue open.

By contrast to the early phylogeographic investigations, some later studies incorporating samples from natural populations found no evidence of recent gene flow between Borneo and Sumatra, and indicated greater differentiation of Bornean populations than previously observed. In the first study to sample widely across geographical locations in Borneo and to include wild orangutans in addition to captive individuals, Warren and colleagues (2001) examined mtDNA variation and compared it to that of a few Sumatran specimens. They found a clear distinction between Bornean and Sumatran orangutans, with an estimated divergence at 1.1 ma. Furthermore, all Bornean haplotypes were found to cluster together in the mtDNA phylogenetic tree, arguing against recent dispersal between the islands. Despite the monophyly of Bornean haplotypes, however, the authors found high levels of diversity between geographical clusters, and interpreted these as evidence for a smooth history of expansion across Borneo without severe genetic bottlenecks. In this scenario, orangutans spread throughout the island of Borneo and then underwent

allopatric divergence as a result of the isolation through geographical barriers. The geographical clusters agreed with the distinction of subspecies through morphological characteristics (Warren *et al.* 2001). Additional support for this scenario was provided by Jalil et al. (2008), who expanded the sample set by incorporating a natural population of Bornean orangutans from a new geographical location in Borneo.

Other authors have, however, advocated a more eventful and punctuated history within Borneo. The findings by Kanthaswamy et al. (2006) of lower levels of mitochondrial variation in Bornean compared to Sumatran orangutans were interpreted as evidence for a recent colonization of Borneo followed by founder effects and genetic bottlenecks. That Borneo and Sumatra shared haplotypes despite high nuclear divergence between them was explained as either an outcome of human translocations or as Muir (2000) proposed, recent dispersal from Borneo to Sumatra. Also supporting a Bornean evolutionary history marked by drastic changes, Steiper (2006) found evidence for a recent Bornean population expansion ca. 39 – 64 ka in analyses using mitochondrial and nuclear regions. The expansion was attributed to either recovery after the devastating effects of the Toba super-eruption or as a result of habitat changes linked to the Pleistocene climatic oscillations.

The role of geographical barriers

So far, only one study has investigated the role of topographical features as natural barriers to orangutan dispersal. Goossens and colleagues (2005) focused on the large Kinabatangan river (Sabah), using a set of 14 nuclear microsatellite markers to examine regional fine scale genetic structuring of populations across and along the same river side. The higher differentiation of populations across the river compared to populations on the same side indicated that the river is indeed a pivotal geographical barrier. This result supports the general role ascribed to the paleo-rivers in shaping the early dispersal of orangutans from the mainland into Borneo and Sumatra. Nevertheless, the effects of such barriers have not been explored at an island-wide level, and thus the implications for species range expansions are still open to debate.

Summary

In sum, the literature contains conflicting evidence for different scenarios of the demographic histories of orangutans, and only a sketchy picture of current population structure. Although all studies so far converge in finding high genetic divergence between the islands, some studies show instances of haplotype sharing and suggest that there has been recent gene flow between Borneo and Sumatra, while other investigations indicate deeper vicariance. Furthermore, the effects of major environmental and geological factors in Sundaland, including the Pleistocene climatic fluctuations, volcanic eruptions and geographical barriers, on the colonization histories and current population structures of orangutans remain a conundrum.

1.5 The effects of sex-biased dispersal

Why sex-biased dispersal matters

Although environmental processes can affect the spatial distribution of populations, resulting in range expansions or contractions, the spatial distribution of genes also changes through individual movement from one population to another in each generation. In vertebrates, individual movement is often in the form of sex-biased dispersal, whereby one sex is more prone to leaving or travelling longer distances from the natal range to breed (Lawson Handley & Perrin 2007). Such individual dispersal is a crucial life history trait that has effects on the species dispersal abilities, population genetic structure and social structure. Let us examine these in turn.

First, sex-biased dispersal has an effect on the speed of a spatial range expansion (Kokko & Lopez-Sepulcre 2006; Jerina & Adamič 2008). Although overcrowding of the habitat can lead to some long-distance dispersal by the sex exhibiting site fidelity or philopatry (Swenson *et al.* 1998; Perrin & Goudet 2001), this effect might not be as critical as short-distance dispersal from the peripheral areas of a population. Indeed, a detailed study of brown bear (*Ursus arctos*) expansion suggests that the rare long-distance dispersal of otherwise philopatric females do not contribute substantially to expansion speed or establishment of new stable populations (Jerina & Adamič 2008). Furthermore, an empirical investigation shows that sex-biased dispersal, by affecting the operational sex-ratio at the leading edge of the expansion, can accelerate or decelerate the rate of this expansion (Miller *et al.* 2011).

Second, sex-biased dispersal has a large impact on population structure. The high relatedness of members of the philopatric sex, combined with the limited gene flow associated with sex-biased dispersal, leads to population subdivision. This subdivision affects effective population size (N_e), and the rate of loss of genetic diversity, which is inversely proportion to N_e (Sugg *et al.* 1996; Storz 1999; Whitlock 2001). The more philopatric sex is expected to show a greater geographical apportioning of genetic diversity. Consequently, in the event of habitat fragmentation or destruction followed by local extinctions, the genetic diversity present in the philopatric sex is more vulnerable to elimination. Particularly relevant to the conservation of endangered species, we can utilize information on social organization to conduct more powerful predictive modeling of loss of diversity under different scenarios (Avise *et al.* 1987), especially relevant given imminent climatic change and ongoing anthropogenic deforestation. Another way in which information of social organization can be used in conservation management is through its consideration in translocation programs or in the establishment of corridors between fragmented pockets of surviving populations, as recommended for orangutans and other species (Bruford *et al.* 2010). Ideally, a translocation program would assess the benefits of mixing individuals of the philopatric sex, which would help reduce the risk of loss of genetic diversity partitioned across populations, and the costs of disrupting kin structures, which could have negative impacts on nepotistic benefits (Avise *et al.* 1987). For instance, in species with female philopatry, translocating an unrelated female within the ranges of a matrilineal cluster might result in reduced tolerance as well as reduced fitness, making it less successful than the translocation of members of the dispersing sex.

Third, no understanding of social structure is complete without knowledge of the relatedness patterns among interacting individuals. Who remains in the natal area and

who leaves has important consequences for the associations of individuals, and whether kin selection accounts for at least some of these. This interest in kin selection has channeled investigation to species with distinct social groupings, particularly those in which social behaviors such as cooperative breeding and coalitions are found. However, less attention has been devoted to non-gregarious species due to their seemingly lower levels of sociality, and because of the difficulties in determining the boundaries of "social networks" (Richard 1985). Fewer investigations also mean that in fact, levels of sociality in such species may be underestimated. Overall, it becomes clear that the opportunities for (cryptic) kin selection have hardly been explored in non-gregarious species. Orangutans for instance, despite their notorious non-gregarious reputation, do interact with preferred partners (Singleton & van Schaik 2002; Mitra Setia *et al.* 2009). In Sumatra, where orangutans show higher levels of sociality compared to Borneo, striking findings of preferential association among physically similar females overlapping in their home ranges were made (Singleton & van Schaik 2002). These observations pointed to clusters of related females, or female kin structures, underpinning the social organization of orangutans. The lack of confirmation of genetic relatedness among these females, however, renders the issue as yet unsettled, but opens an exciting avenue of research to determine whether kin structures are indeed common in orangutan populations.

Fourthly and finally, insights into the social organization and structure of orangutans might enable us to reconstruct the ancestral state for the great apes. Genetic and behavioral evidence show that, in contrast to most other mammals, the African great apes and humans are not characterized by a simple pattern of female philopatry and male dispersal, but instead share a tendency toward female dispersal. In fact, male cooperation and male philopatry are notable features of chimpanzee social organization (Langergraber *et al.* 2007), while bonobos also show male philopatry (Eriksson *et al.* 2006). In gorillas males and females have been found to emigrate, although dispersal distances between the sexes vary considerably (Douadi *et al.* 2007). In humans, hunter-gatherer societies show varied patterns but male patrilocality and female-biased dispersal is highly widespread in current populations (Van Horn *et al.* 2004; Lawson Handley & Perrin 2007). Yet, the characterization of the dispersal patterns in orangutans and the implications for the contrasting patterns among the other great apes are still to be settled.

Insights into contemporary patterns of sex-biased dispersal in orangutans

In orangutans, the signature of historical sex-biased dispersal on phylogeographic patterns has so far not been explored. As for contemporary patterns, there have been behavioral and genetic studies, but these have, quite surprisingly, yielded incongruent results. Evidently, both types of measures are required to unravel the dispersal patterns of orangutans. In particular, while behavioral data collection has the advantage of being a direct method that provides rich individual information, the movement of individuals in space does not necessarily translate to gene flow, and thus into effective dispersal (Prugnolle & de Meeus 2002). Furthermore, there are inherent logistical difficulties in obtaining complete long-term behavioral information from non-gregarious species such as orangutans with large, overlapping home ranges and an arboreal lifestyle. Finally, observational evidence at single sites necessarily relies on small sample sizes. So the combination of behavioral and genetic methods across sites should produce the most complete and accurate picture (Lawson Handley & Perrin 2007).

To date, behavioral observations from several long-term field sites all converge in suggesting that orangutans show the predominant dispersal pattern in mammalian species, whereby females remain close to their mother's home ranges and males disperse to breed (Galdikas 1985b; van Schaik & van Hooff 1996; Singleton & van Schaik 2002). Interestingly, and in support of such a pattern, the adult female descendants of a rehabilitant female at the Sumatran field site of Ketambe were observed to settle near the maternal range (Mitra Setia *et al.* 2009), whereas males tended to disappear (van Schaik & van Hooff 1996). Nevertheless, rare instances where mature males were seen in the ranges of their reproductively active mothers have also been noted (van Noordwijk *et al.* 2009).

By contrast to the behavioral observations, genetic studies of relatedness have been very scarce and produced conflicting results. The only three studies to date have all compared the average pairwise relatedness of females and males using biparentally inherited autosomal markers. These markers provide a snapshot of dispersal within one generation, also referred to as instantaneous dispersal, since recombination occurs in the next generation (Prugnolle & de Meeus 2002). In the first of these studies, Utami et al. (2002) found that males and females displayed similarly negative relatedness, and argued that both sexes were equally likely to disperse. However, the potentially confounding inclusion of rehabilitant females and their descendants was highlighted by the authors. In a second study focusing on a natural population in Sabah (Borneo), Goossens et al. (2006b) found that the females and males all showed similarly positive relatedness. This finding was taken as evidence for philopatry in females as well as males, although the authors remarked on the obstacles to dispersal posed by severe habitat fragmentation of the area in question. Finally, the third study, which has only recently been published, showed that in the natural population of Sabangau (Borneo), relatedness levels were significantly higher among the five females compared to the eleven males investigated (Morrogh-Bernard *et al.* 2010). While this last study agrees with most field observations, sample size was small, implying that slight biases in relatedness estimates could have a large impact on the results and interpretations thereof.

Summary

Sex-biased dispersal has a profound influence on the partitioning of genetic diversity, and consequently, on the impact of large-scale environmental changes and drift on the maintenance and loss of this genetic diversity. Hence, uncovering patterns of sex-biased dispersal provides insights into population dynamics. While behavioral observations indicate that orangutans have a social organization characterized by female philopatry and male-biased dispersal, genetic studies of instantaneous (one-generation) dispersal have suggested different patterns across sites. The discrepancies pose a riddle to biologists seeking to understand orangutan life history.

1.6 Aims of the thesis

This thesis seeks to assess the impact of different evolutionary mechanisms in structuring orangutan genetic diversity as part of a larger ongoing project. The present study focuses on Bornean orangutans, while another doctoral study is devoted to the Sumatran orangutans. The synergetic output of both studies will be integrated to produce a complete picture on the genetic diversification of orangutans.

With a focus on Bornean orangutans, this study seeks to attain two main aims:

1. **To assess the impact of environmental and biological forces on the phylogeography and population genetic structure of Bornean orangutans, focusing particularly on historical gene flow.** A major endeavor is to uncover whether historical patterns of dispersal and gene flow in orangutans were shaped by Pleistocene climatic changes, major volcanic eruptions, riverine barriers and sex-biased dispersal patterns that left a marked genetic signature.
2. **To resolve the question of sex-biased dispersal and relatedness patterns in orangutans, focusing on contemporary gene flow**. The previous aim addresses the potential historical effects of sex-biased dispersal on orangutan population genetic structure. Nevertheless, current patterns and their effects, which might differ from past patterns, are unclear due to the equivocal results of recent genetic studies on instantaneous (one-generation) dispersal. This aim is investigated through the following studies:
    a. **In-depth investigation of sex-biased dispersal through a case study**, capitalizing on the extensive spatial, behavioral and genetic information available at one of the sites. This study highlights the potential ecological and particularly social benefits that may drive sex-specific philopatry. These are important because, by driving the pattern of dispersal, they shape the genetic structure of populations and affect diversification. Hence, this study emphasizes the importance of clarifying the social organization of orangutans.
    b. **Examination of sex-biased dispersal across orangutan populations**, with an assessment of different genetic methods. The comparison should allow us to assess current conventional genetic measures of instantaneous dispersal, through reference to the case study, and to determine whether the discrepant patterns found to date reflect intra-specific variation or are the outcome of methodological issues.

To address these goals, it is important to first obtain reliable genetic data from the different populations, through the application of a suitable sampling strategy and appropriate molecular techniques. The section that follows details the methodological considerations concerning these two important aspects that lay the groundwork of the analytical work.

1.7 Methodological considerations

1.7.1 Sampling strategy

With a view to characterizing the genetic diversity across and within orangutan populations, several sampling criteria were taken into account. First, in order to uncover the distribution of genetic diversity across orangutan populations, the systematic sampling of individuals from a) natural populations and b) throughout the entire ranges of the species are necessary. Despite major logistical difficulties associated with sampling an arboreal and elusive species, this point is critical in order to obtain reliable results. Second, to disentangle how genetic diversity is structured by sex-biased dispersal, the exhaustive sampling within populations is crucial to reduce biases stemming from small sample sizes. Third, behavioral data is important as its combination with genetic data should produce a more complete picture. These criteria were met through the

establishment of an extensive and fruitful collaboration with other researchers working at long-term field sites or involved in the sampling of orangutans.

1.7.2 Molecular tools

The application of molecular tools to tackle questions on demographic history, population genetic and socio-genetic topics has burgeoned since the advent of polymerase chain reaction (PCR). Indeed, the possibility to exponentially amplify DNA from samples containing minute amounts has opened a new window of opportunity. Coupled with ongoing forensic advances, recent molecular techniques are becoming increasingly useful especially in the study of endangered species, for which there is a strict enforcement of non-invasive methods. Thus, researchers are no longer restricted to the usage of blood samples from zoo or rehabilitant individuals, with the associated provenance uncertainty, but can also use lower quality and quantity DNA samples such as hair and feces from individuals of natural populations. The study limitations therefore are set by the extent of sampling of natural populations that is possible. Some genetic markers that have been successfully applied using non-invasively collected samples are uniparentally inherited markers such as those on the mitochondrial DNA (mtDNA) and the Y chromosome, and biparentally inherited markers such as microsatellites. While mitochondrial DNA is found as hundreds to thousands of copies in the cell, Y-chromosomal markers and microsatellite loci are nuclear, and thus found as single copies, making them more difficult to target. Furthermore, suitable species-specific markers on the Y chromosome are not easily found, although greater investment in this research area should be very fruitful (Greminger *et al.* 2010). For this reason, studies that use mtDNA are more abundant, but application and combination with the other markers is finally on the increase. For this thesis, I have focused on the mtDNA and a large set of nuclear microsatellite markers, as Y-chromosomal markers were initially not available for amplification in orangutans and have only recently been the scope of a recent MSc. thesis. The following sections expand on these genetic markers and the issues pertinent to their application to non-invasive samples.

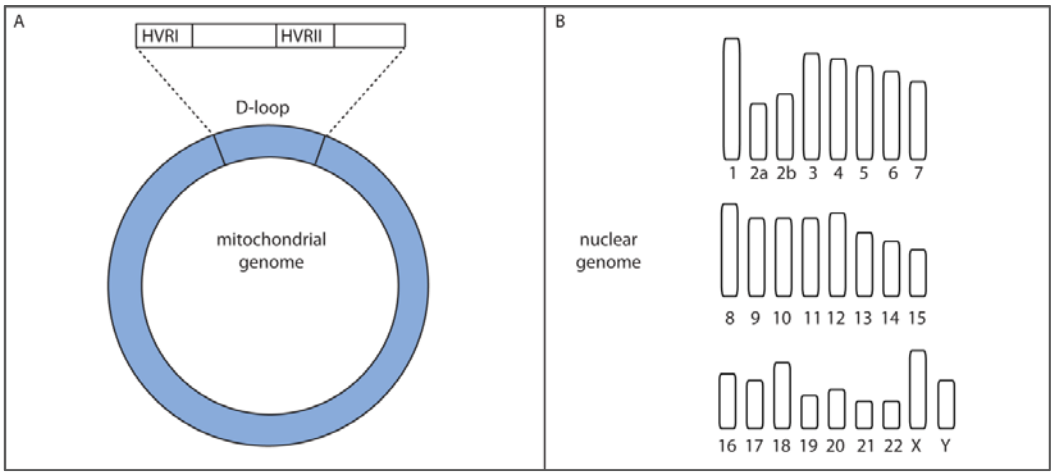
Mitochondrial DNA

The circular mitochondrial genome stretching approximately 16.6 kilobases (kb) (Fig. 1.3A) is contained within the energy pumping organelles of the cell, the mitochondria. On average, four to five mitochondrial genomes are contained within one mitochondrion. Since cells may have hundreds of mitochondria, they can contain hundreds and even thousands of copies of mtDNA. Such copiousness favors its application in degraded and trace samples (Butler 2005). Furthermore, the maternal transmission mode and absence of recombination of mtDNA, as well as the high mutation rate compared to nuclear markers, renders it an excellent marker to investigate the genealogical history of the female line (Butler 2005; Torroni *et al.* 2006). It must be noted that although cases of paternal transmission and recombination have been reported, they have failed to produce convincing evidence (Pakendorf & Stoneking 2005). One caveat of the lack of recombination of the mitochondrial genome is that selection of certain alleles of a specific gene will inevitably lead to the "hitchhiking" of the rest of the sequence, even if it has no selective advantage (Handley et al., 2007, Pilkington et al., 2008.

Since non-invasive samples such as hair and feces contain low quantities of degraded DNA that are shortened in length, generally only small selected regions of the entire

mitochondrial genome are usually "copied" or amplified, and sequenced. Some of the most widely used mtDNA sequences are those of the hypervariable region (HVR), found within the control or displacement loop (D-loop) region. Two of these are generally used: the HVR I and the HVR II. These DNA sequences have the advantages of being non-coding and subject to a high mutation rate, estimated in humans through phylogenetic studies to fall in the range 0.075–0.165 x 10^{-6} substitutions/site/year (Pakendorf & Stoneking 2005). One problem, however, is the presence of translocated mtDNA sequences in the nuclear genome, or "numts", which represent separately evolving paralogous copies. The incorporation of these in an mtDNA phylogeny would lead to erroneous inferences. Nevertheless, a previous study found no evidence for "numts" of the HVR I region in orangutans (Thalmann *et al.* 2004). Given the advantages of the region for population genetic analyses, the present study capitalizes on the usage of the HVRI to trace female evolutionary histories and examine maternal co-ancestry in orangutans.

Fig. 1.3 Schematic illustration of the mitochondrial and nuclear genomes. A) Mitochondrial genome and the HVR I and II regions of the D-loop, based on Butler (2005); B) Nuclear genome of *Pongo abelii*, based on Ensembl (2011).



Autosomal microsatellite markers

The nuclear genome, illustrated in Fig.1.3B, contains a large number of repetitive elements, including microsatellite DNA. These markers are short tandemly repeated sequences with repeat units between 2-6 base pairs (bp) in length. Polymorphic microsatellite markers differ in the total number of repeat units, and due to the high variation among individuals they are very useful for purposes of identification of individuals. Furthermore, their high mutation rate makes them suitable for studies of recent population histories.

For this study, a series of tetranucleotide and dinucleotide repeat markers isolated in human as well as orangutan autosomes were used. The human-derived markers have been successfully applied to orangutans in previous studies (Goossens *et al.* 2005), and have therefore been used in the present endeavor for comparative purposes. Additionally, because markers specific to the species in question are expected to show higher levels of polymorphism, resulting in greater informativeness, *Pongo*-specific markers were developed in our laboratory (Nietlisbach *et al.* 2010).

Non-invasive samples present some problematic issues associated with low DNA quality and quantity, which could lead to erroneous genotypes. Some problems include the systematic failure to amplify one of the alleles of a heterozygous locus, resulting in allelic dropout, the misinterpretation of contaminating DNA as an allele, resulting in a false allele, or the misinterpretation of a non-template allele known as a stutter band which occurs due replication slippage (Morin *et al.* 2001a; Morin *et al.* 2009). To counter these issues and maximize genotype reliability, we followed the protocol recommended by Morin et al. (2001b). This approach involved DNA quantification through real-time PCRs to determine the number of positive PCR replicates per extract necessary to obtain a 99% confidence level that a homozygous genotype is correct. For a heterozygous genotype, our criterion was the observation of each of the two alleles at least twice in independent PCRs.

1.8 Structure of the thesis

The main body of the thesis is structured into a series of chapters that have either been published as papers or are in preparation for submission. These chapters are complemented by the general introduction, the general discussion, and a set of co-authored publications. Brief synopses for these chapters are provided below.

CHAPTER 2 (published)

N. Arora, A. Nater, C. P. van Schaik, E. P. Willems, M. A. van Noordwijk, B. Goossens, N. Morf, M. Bastian, C. Knott, H. Morrogh-Bernard, N. Kuze, T. Kanamori, J. Pamungkas, D. Perwitasari-Farajallahi, E. Verschoor, K. Warren, M. Krützen. (2010). Effects of Pleistocene glaciations and rivers on the population structure of Bornean orangutans (*Pongo pygmaeus*). *Proceedings of the National Academy of Sciences U S A,* 107**,** 21376-81.

Synopsis: Addressing the first aim (1), this chapter delves into the processes that shaped historical patterns of gene flow focusing on the role of the major environmental mechanisms in the dynamic region of Sundaland as well as sex-biased dispersal.

CHAPTER 3 (in preparation)

N. Arora[1], E. P. Willems[1], M. A. van Noordwijk[1], C.P. van Schaik[1], C. Ackermann[1], M.Greminger[1], A. Nater[1], L. P. Dunkel[1] , and M. Krützen[1] (2011). Spatial and genetic evidence for matrilineal clusters and male-biased dispersal in a population of Bornean orangutans (*Pongo pygmaeus*). To be submitted to *Molecular Ecology*

Synopsis: Having examined the environmental and biological factors that determined historical patterns of gene flow and structured orangutan genetic diversity, this chapter zooms in on the spatio-temporal scale to tackle the second aim (2a): the focus now is on obtaining a snapshot of current dispersal patterns at a population that allows integration of extensive spatial, behavioral and genetic data is available. This case study emphasizes the interest in unraveling the relatedness among individuals at the site, in view of the possible social benefits driving sex-specific philopatry and dispersal, and thus, influencing population genetic structure.

CHAPTER 4 (in preparation)

N. Arora[1], C. van Schaik[1], M. van Noordwijk[1], A. Nater[1], N. Morf[1], C. Ackermann[1], M. Greminger[1], B. Goossens[2], J. Pamungkas[3], D. Perwitasari-Farajallah[3], M. Bastian[4], C. Knott[4], H. Morrogh-Bernard[5], N. Kuze[6], T. Kanamori[7], Jinliang Wang[8] and M. Krützen[1] (2011). Emerging patterns of sex-biased dispersal in orangutans. To be submitted to *Molecular Ecology*

Synopsis: Following the detailed case study of relatedness and dispersal at one study site, this chapter further addresses the second aim (2b) through a comparison of dispersal patterns at several Bornean and Sumatran study sites. The current methodologies available for genetic analyses of relatedness and dispersal are evaluated, with a focus on their application to non-gregarious species such as orangutans.

CHAPTER 5

General discussion

Synopsis: The chapter contains succinct summaries of the main findings of each study chapter. These are followed by a broader discussion placing the findings in a contextual framework, outlining some of the limitations, and briefly outlining the opportunities for future work.

CHAPTER 6

Co-authored publication and manuscript abstracts

Synopsis: In this chapter, the abstracts of a series of relevant co-authored publications are presented. The journal reprints are collated in Appendix III.

1.9 Author contributions

The goals set out in this thesis were achieved through work I did including research design, laboratory work, statistical analyses, manuscript writing, application for additional funding, and supervision of interns, students and a lab assistant. Other tasks were devoted to setting the groundwork for the main investigation, but are not reflected herein. For instance, for two field sites considerable effort was devoted to the identification of the individuals observed at different time periods. Additionally, I had the opportunity to engage in side projects, including the identification of geographical provenance for rehabilitant individuals returned to Indonesia from Thailand.

This thesis and the papers would not have been possible without the contributions of a well-integrated team. My advisors Michael Krützen and Carel van Schaik were not only vital in obtaining funding, designing research and developing ideas, but were also closely involved in the development of the research stages, and the final stages of writing for all the papers in the thesis. Maja Greminger, Alex Nater, Erik P. Willems, and Maria van Noordwijk reviewed manuscripts in detail and provided highly valuable comments, prompting many of the analyses carried out. Erik P. Willems contributed with extensive GIS and spatial analyses, also producing the GIS maps of orangutan distribution that are being used in many of our genetics papers. Alex Nater, Maja Greminger and Nadja Morf

also conducted laboratory work to generate raw data for some of the populations examined. Both Maja Greminger and Alex Nater gave invaluable theoretical and technical advice. Alex Nater also aided in the lengthy identification analyses carried out for the different males at the study site of Tuanan, enabling their distinction. For this site, Maria van Noordwijk and Lynda Dunkel aided with extensive and detailed behavioral information and supportive feedback. Benoit Goossens collaborated closely, providing genotypic data for the Lower Kinabatangan Wildlife Sanctuary, and together with Marc Ancrenaz facilitating behavioral data. Through the grants obtained from the A.H. Shultz Foundation, the lab assistance ship of Corinne Ackermann became possible, who painstakingly carried out the processing of newly available samples, as well as the completion and proofing of raw data, and supervision of an MSc. Student. Kristin Warren and Ernst Verschoor contributed with the DNA extracts used in their previous publication. Jinliang Wang produced the software used for analyses of relatedness and provided highly constructive feedback. Benoit Goossens, Maria van Noordwijk, Helen Morrogh-Bernard, Meredith Bastian, Cheryl Knott, Noko Kuze, Tomoko Kanamori, Joko Pamungkas, and Dyah Perwitasari-Farajallah were all involved in the field observations at long-terms study sites and/or sample coordination, which was critical to attain the dataset used herein.

2 Effects of Pleistocene glaciations and rivers on the population structure of Bornean orangutans (*Pongo pygmaeus*)

2.1 Abstract

Sundaland, a tropical hotspot of biodiversity comprising Borneo and Sumatra among other islands, the Malay Peninsula and a shallow sea, has been subject to dramatic environmental processes. Thus, it presents an ideal opportunity to investigate the role of environmental mechanisms in outlining species distribution and diversity. We investigated the population structure and underlying mechanisms of an insular endemic, the Bornean orangutan (*Pongo pygmaeus*). Phylogenetic reconstructions based on mtDNA sequences from 211 wild orangutans covering the entire range of the species indicate an unexpectedly recent common ancestor of Bornean orangutans 176 ka (95% highest posterior density (HPD) 72-322 ka), pointing to a Pleistocene refugium. High mtDNA differentiation among populations and rare haplotype sharing is consistent with a pattern of strong female philopatry. This is corroborated by isolation by distance tests, which show a significant correlation with mtDNA divergence and distance and a strong effect of rivers as barriers for female movement. Both frequency-based and Bayesian clustering analyses using up to 25 nuclear microsatellite loci revealed a significant separation among all populations, as well as a small degree of male-mediated gene flow. This study highlights the unique effects of environmental and biological features on the evolutionary history of Bornean orangutans, a highly endangered species particularly vulnerable to future climate and anthropogenic change as an insular endemic.

2.2 Introduction

Environmental mechanisms are some of the most important forces affecting the evolutionary history and current distribution of species. Such mechanisms have been invoked to explain genetic structure in many temperate European and North American species but with little focus on hotspots of biodiversity and endemism in the tropics (Hewitt 2004), where the forces underlying patterns of genetic diversity and differentiation are especially intriguing.

The tropical Asian hotspot of Sundaland is remarkable in that it has been subject to dramatic geological and environmental changes (Hall 2002; Bird *et al.* 2005). This now partly submerged continental shelf encompasses the Malaysian peninsula, the islands of Borneo, Sumatra, Java and possibly Palawan (Bird *et al.* 2005). It is a historically dynamic tectonic area that underwent notable landmass configuration changes (Hall 2002). More recently, it has been severely affected by the Pleistocene climatic oscillations (Verstappen 1997) of the Quaternary. Changes in sea levels resulted in the cyclical exposure of the continental shelf and the formation of land bridges between the islands (Verstappen 1997; Voris 2000), allowing for species interchange with subsequent isolation (Fordham & Brook 2010). Moreover, climatic fluctuations were accompanied by vegetation changes (Verstappen 1975; Heaney 1991; Bird *et al.* 2005), with shifts in the range and elevational distribution of rainforests. Thus, these changes led to habitat expansions or contractions, leading to new openings or barriers to gene flow. The

Pleistocene was further punctuated by intense regional climatic and habitat changes through extraordinary volcanic eruptions, especially of Mount Toba (Rampino & Self 1992; Williams *et al.* 2009). Finally, Sundaland contains many interesting topographical features, including rivers, lakes and mountains (Rijksen & Meijaard 1999; Voris 2000; Harrison *et al.* 2006) that may have acted as barriers to dispersal for a number of species, adding yet another potential allopatric force.

The roles of these environmental forces in driving biotic diversity and endemism remain underexplored, particularly in Borneo, the world's second largest tropical island as well as the easternmost Sunda region abutting Wallace's line (Moss & Wilson 1998; Slik *et al.* 2003). Its unusually high species endemism (MacKinnon *et al.* 1996; Moss & Wilson 1998; van Welzen *et al.* 2005) suggests a combination of specialized ecological niches, refugia formation and long periods of isolation.

Among the species endemic to the island are the Bornean orangutans (*Pongo pygmaeus*). This rainforest canopy-bound species with an unusually slow life history is characterized by a rich spectrum of genetic, morphological and cultural differences (Delgado & Van Schaik 2000; Warren *et al.* 2001; van Schaik *et al.* 2003). Fossils indicate a much wider distribution of orangutans during the Pleistocene extending from Southern China and Vietnam to Java (Delgado & Van Schaik 2000; Harrison *et al.* 2006), but orangutans are currently only found, as distinct species, in Borneo (*P. pygmaeus*) and Sumatra (*P. abelii*). The ancestors of orangutans therefore probably migrated from the mainland to Sumatra and from there to Borneo (Rijksen & Meijaard 1999), yet it remains unclear when and how these colonization events took place.

It is also unclear how the exceptional environmental features of Sundaland, combined with the characteristic behavioral and ecological traits of orangutans, have shaped their phylogeography. For instance, isolation in refugia or through riverine barriers have been described as important forces underlying the genetic structure of some of the African great apes (Eriksson *et al.* 2004; Gonder *et al.* 2006; Anthony *et al.* 2007), yet the evolutionary history of orangutans remains unresolved. Firstly, the high genetic differentiation between Bornean and Sumatran orangutans (Xu & Arnason 1996; Warren *et al.* 2001) is intriguing given recurrent land bridge formation between the islands during the Pleistocene glacial periods (Voris 2000). Secondly, within Borneo, arguments for a stable distribution since colonization (Muir *et al.* 2000) clash with that of a bottleneck possibly associated with the last eruption of Mount Toba (Steiper 2006). Thirdly, the three Bornean subspecies (*P.p.pygmaeus, P.p.wurmbii, P.p.morio*), described on the basis of morphological characteristics (Groves 2001), show unexplained genetic substructuring (Warren *et al.* 2001). Fourthly, as for geographical features, the marked role of rivers as dispersal barriers has been highlighted in the study of populations in Sabah (Goossens *et al.* 2005; Jalil *et al.* 2008), but it remains to be seen whether other rivers have had similar vicariant effects. Thus, the relative importance of the Pleistocene sea level and vegetation changes, Toba eruptions and rivers as dispersal barriers, against the background of regular dispersal behavior of orangutans, remains unknown.

These questions also acquire special relevance today from a conservation perspective, in the light of ongoing habitat conversion (Singleton *et al.* 2004) and predicted future climate change (Provan & Bennett 2008; Hofreiter & Stewart 2009), particularly for insular endemics and highly endangered species such as orangutans.

We recently obtained non-invasive wild Bornean orangutan samples from seven long-term study sites, as well as other localities, thus encompassing most of the species' range. Capitalizing on the most extensive sample size to date, we provide genetic evidence for a recent radiation of Bornean populations within the Middle to Late Pleistocene. We further illustrate the role of rivers and sex-biased dispersal in generating the marked population structure of the largest arboreal primate.

2.3 Materials and Methods

Samples and datasets

Our data comprise non-invasively collected fecal and hair samples from a number of long-term study sites: Gunung Palung (GP), Sabangau (SB), Sungai Lading (SL), Danum Valley Conservation Area (DV), and the Lower Kinabatangan Wildlife Sanctuary (Fig. 2.1b). We partitioned the latter site into South Kinabatangan (SK) and North Kinabatangan (NK), given the significant differentiation between the locales found by Goossens et al. (2005). In addition, we incorporated scattered samples from Warren et al. (2001; Table S8), encompassing most of the current distribution of *P. pygmaeus* (Fig. 2.1b). Depending on sample quality and data availability, we used two different datasets for mtDNA analyses, and two for nuclear microsatellite analyses (Table S3). DNA extraction and quantification procedures are described in the SI Text.

MtDNA analyses

Based on unique microsatellite genotypes or mtDNA haplotypes (see SI Text), we obtained the following long-term study site sample sizes: SA (n=23), SL (n=26), TU (n=30), DV (n=18). We also sequenced low DNA quantity samples from GP (n=20), where individual identification was done through long-term observational data. Additionally, haplotypes for individuals from SK (n=38) and NK (n=35) were from Jalil et al. (2008; Genbank Acc. No. EU547189 - EU547201). Finally, we re-sequenced 21 extracts from the Bornean samples in Warren et al. (2001) to cover the same region of mtDNA (SI, Table 2.2). We sequenced a 323 bp region of the mitochondrial DNA (mtDNA) hypervariable region I (HVRI). Details on the primers, PCR conditions and raw data analyses are provided in the SI Text. Summary statistics including haplotype diversity (hd), nucleotide diversity (π) and average pairwise differences were calculated in DNAsp 5 (Librado & Rozas 2009) and Arlequin 3.11 (Excoffier *et al.* 2005). We conducted model selection tests on jModelTest 0.1 (Guindon & Gascuel 2003; Posada 2008), using the Akaike information criterion (AIC) to choose the most suitable model and its parameters.

For the phylogenetic analyses, we incorporated HVRI haplotypes from all long-term study sites as well as Warren's re-sequenced samples (SI, Table 2.2-2.3). First, to infer the coalescence date for Bornean mtDNA haplotypes, we used a Bayesian MCMC analysis as implemented in BEAST 1.5.4 (Drummond & Rambaut 2007) and produced a phylogenetic tree. We included the collapsed haplotypes from 211 Bornean and 6 Sumatran orangutans, as well as 19 humans as an outgroup. Based on the AIC from jModeltest, we selected the HKY + G model. We used an uncorrelated relaxed log-normal clock (Drummond *et al.* 2006), specifying a normal distribution with a mean HVRI substitution rate of 0.1643 substitutions per nucleotide per myr for the mean rate

prior. We chose this corrected HVRI estimate (Soares *et al.* 2009), because it takes into account the effects of purifying selection on the entire mtDNA molecule as well as saturation factors affecting the molecular rate decay described in numerous studies (Ho *et al.* 2005; Pulquério & Nichols 2007; Endicott *et al.* 2009), and is therefore appropriate for population level analyses (Ho *et al.* 2008; Soares *et al.* 2009). The 95% confidence interval for the normal distribution spanned HVRI substitution rates obtained in other studies, from 0.06-0.25 substitutions/site/myr (Santos *et al.* 2005). Using the birth-death prior for branching rates, we carried out two runs for 25 million generations with parameter sampling every 1,000 generations. Tracer 1.4.1 (Rambaut & Drummond 2005) was then used to examine whether the 10% burn-in period and effective sample size were adequate. Both runs were combined in LogCombiner 1.4.8, and the resulting tree visualized and edited using Figtree 1.2 (Rambaut 2008), omitting human haplotypes. Second, to infer the coalescence date for Bornean and Sumatran mtDNA haplotypes, we used the same procedure, but instead of the corrected mutation rate, we chose two fossil based divergence estimates as priors. Fossil calibration points provide estimates of phylogenetic rates suitable for analyses at the interspecific level (Ho *et al.* 2008). The two calibration points were the Ponginae-Homininae divergence around 14 ma (Kelley 2002; Raaum *et al.* 2005) and the Pan-Homo divergence older than 6 ma (Brunet *et al.* 2002; Zollikofer *et al.* 2005). We specified lognormally distributed priors, appropriate for paleontological data (Ho 2007). For the Ponginae-Homininae divergence, we used a lognormal mean of 0, lognormal standard deviation of 0.56 and offset of 13 ma, thereby obtaining a broad distribution with a 95% interval from 13.4-20 ma. This range incorporates the uncertainties associated with the upper bound estimate of a split. For the Pan-Homo calibration, we used a lognormal mean of 0, lognormal standard deviation of 0.56 and offset of 5 ma, spanning a 95% interval from 5.4 to 7.5 ma. The tree topology remained the same as in the first analysis so we do not present it. Third, we investigated phylogenetic relationships at the intraspecific level by generating a median-joining network for the Bornean haplotypes using Network 4.0 (Bandelt *et al.* 1999).

For the population structure analyses, we used data from the long-term study sites GP, SA, SL, TU, DV, NK, SK. In addition, we incorporated sampling sites from Warren et al. (2001) for which at least 3 samples of precise origin are available: Danau Sentarum (DS) and Kutai (KU) (SI, Table 2.2; c.f. Anthony *et al.* 2007). We calculated pairwise Φ_{ST} values in Arlequin, using the Tamura Nei model (Tamura & Nei 1993) and a gamma distribution shape parameter of 0.344. We obtained significance levels using 10,000 permutations. To define the most differentiated groups of populations, we also performed a spatial analysis of molecular variance with SAMOVA 1.0 (Dupanloup *et al.* 2002), using previously published geographical coordinates (Warren *et al.* 2001; Wich *et al.* 2009b).

Microsatellite analyses

Microsatellite analyses focused only on samples from long-term study sites GP, SB, SL, TU, DV, SK and NK. For the low DNA quality and quantity samples from GP we could obtain genotypes for 6 individuals. We genotyped samples from all sites except SK and NK using a panel of 25 highly polymorphic nuclear microsatellite markers (Goossens *et al.* 2005; Nietlisbach *et al.* 2010) listed in SI, Table 2.4, following the protocol given in the SI Text. Additionally, we incorporated previously generated data from NK and SK for 12 microsatellite markers (Goossens *et al.* 2005), which were part of our panel of 25 markers. We standardized the data and performed identity analyses as described in SI

Text. After this procedure, we obtained two data sets: (i) Dataset I comprises 25 markers and 98 individuals from five study sites GP (n =6), SA (n =19), SL (n=26), TU (n=29), and DV (n=18); (ii) Dataset II comprises 12 markers and 295 individuals from seven study sites, including all from Dataset I plus NK (n=91) and SK (n=106).

After Bonferroni correction, we found no deviation from HWE, and only four pairs of different loci from two populations showed LD, which is most likely explained by demographic effects rather than linkage. Also, we found evidence for possible null alleles for one locus in one population (SI, Table 2.7). Since it was not consistent across populations, we did not exclude this locus from further analyses.

We used Genetix 4.05 (Belkhir *et al.* 1996-2004) to obtain population pairwise F_{ST} values and significance levels. We also performed two separate analyses on STRUCTURE 2.3 (Pritchard *et al.* 2000), using the admixture model with correlated allele frequencies, and the Locprior model, which improves clustering when the signal is weak without spuriously inferring structure if absent (Hubisz *et al.* 2009). We specified a burn-in length of 10^5 followed by 10^6 MCMC steps. For each K we ran the analysis 10 times. In the first analysis we incorporated the widely distributed 7 populations genotyped at 12 microsatellite markers (Dataset II). In the second analysis, we further refined our findings focusing on the 5 populations for which we have genotypes for 26 microsatellite markers (Dataset I).

We calculated geographic distance matrices as Euclidean and cost path distances between all study populations. The latter, representing true surface distances circumnavigating riverine barriers, were computed from the Shuttle Radar Topography Mission (SRTM) global Digital Elevation Model (DEM), as distributed by ESRI (2008b). We clipped the DEM to encompass the whole of Borneo and filled sinks to obtain a depressionless elevation model, which was then reprojected into the Universal Transverse Mercator coordinate system with a resolution of 100 meters. From this, we constructed a flow accumulation raster and extracted grid cells with values ≥ 1000 to generate a stream order raster following Strahler's (1957) convention. We then produced a cost raster by designating areas with flow accumulation values < 1000 and streams of order 1-2 a cost of 1, whereas streams of order 3, 4 and 5 were assigned a cost of 3000, 4000 and 5000 respectively. Streams of order 6-7 were designated as un-crossable barriers (c.f. Anthony *et al.* 2007). After masking the resulting cost raster with the SRTM Water dataset (ESRI 2008b), we calculated dyadic cost path distances between the study populations. For all geospatial analyses, we employed ArcInfo's Spatial Analyst extension for ArcGIS 9.3 (ESRI 2008a).

To investigate the association between genetic (pairwise Φ_{ST} for HVRI and F_{ST} for microsatellite markers) and geographical distances (Euclidean and cost path), we performed (partial) Mantel tests in R 2.10.1 (R_Development_Core_Team 2009), using the 'ecodist' package (Goslee & Urban 2007).

2.4 Results

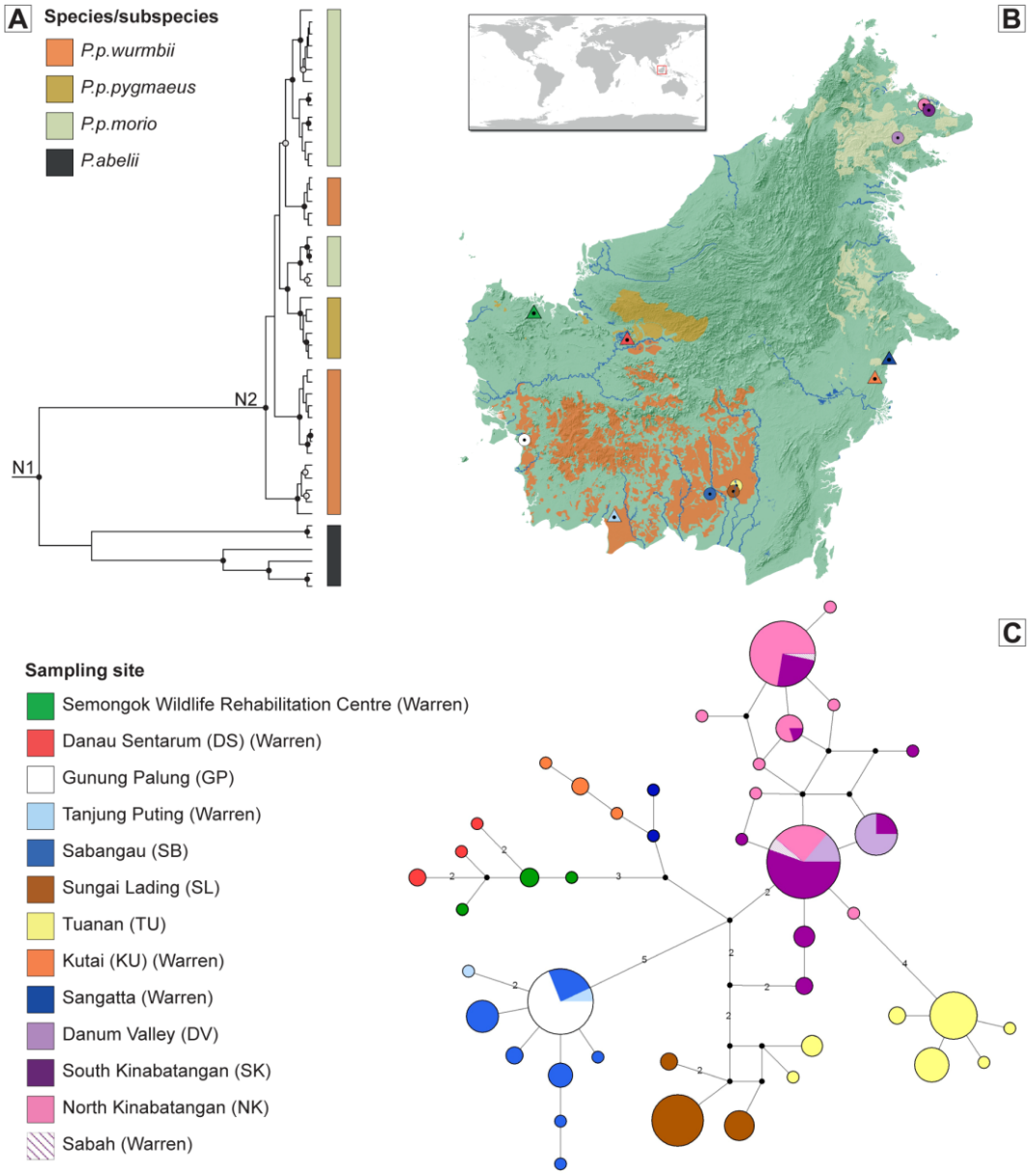
MtDNA analyses

We generated a phylogenetic tree for the mitochondrial (mtDNA) haplotypes from 211 individuals distributed throughout 10 sampling sites in Borneo (Fig. 2.1b), including 6

Sumatran individuals. The tree (Fig. 2.1a) shows a monophyletic Bornean clade with a surprisingly recent mean coalescence date of 176 ka (95% highest posterior density (HPD) 72-322 ka), contrasting with a much older estimate from a previous study (Warren *et al.* 2001). The phylogenetic tree and divergence estimate further illustrate the deeper coalescence of Bornean and Sumatran haplotypes (mean 3.6 ma, 95% HPD 2.3-5 ma). Given the recurrent formation of potential connections between the islands, these findings point to an unexpectedly recent and single origin for current Bornean populations. Furthermore, the Bornean subspecies, as currently recognized on the basis of morphological characteristics, are not reciprocally monophyletic, and should therefore be reconsidered.

The surprisingly recent radiation of a single Bornean lineage calls for a more detailed exploration of Bornean phylogeography. We generated an mtDNA phylogenetic network (Fig. 2.1c), more appropriate for population level studies than phylogenetic trees since they do not force possible ancestral haplotypes to the tips (Posada & Crandall 1998; Posada & Crandall 2001). The network revealed seven main star-like geographic clusters, reflecting considerable structuring within the different subspecies. These seven clusters were further supported by a spatial analysis of molecular variance (SAMOVA), which defines groups of populations that are "geographically homogeneous and maximally differentiated from each other" (Dupanloup *et al.* 2002). The analysis indicated that among-group variance asymptotes at 79.27% (F_{CT} =0.793, p<0.01) with 7 groups of populations. The grouping corresponds to an almost complete separation of all sampled sites except for: i) Danum Valley (DV), which clusters with South Kinabatangan (SK), a site in close proximity (~90 km) not separated by geographical barriers (Fig.2.1b); and ii) Gunung Palung (GP), clustering with Sabangau (SA), a site with which it shares its only haplotype. Our results point to strong inter-population differentiation for mtDNA, as corroborated by the high and significant Φ_{ST} values for all 36 population pairs (Table 2.1). The exceptions are three lower, albeit still significant Φ_{ST} values between the sites that share haplotypes. Given the heavy reliance of Φ_{ST} and other classic moment-based estimators on intra-population diversity (Jost 2008), we also computed population average pairwise differences (SI, Table 2.1). We found generally higher levels of diversity between populations than within, providing additional support for inter-population differentiation.

Fig.2.1 Phylogenetic reconstruction and sampling sites of Bornean orangutans. (A) Bayesian phylogenetic tree of Bornean and Sumatran mtDNA haplotypes. Circles show posterior probabilities (> 0.5, open circles; > 0.75, black circles). Colored bars next to tips indicate species/subspecies designation. (B) Map of Borneo with location of sampling sites. Triangles correspond to sites for which only mtDNA are available, circles correspond to sites for which additionally microsatellite data are available. Sites with re-sequenced data from Warren et al. (2001) are indicated in parentheses. Colored ranges on the map represent subspecies. (c) MJ network of Bornean mtDNA HVRI haplotypes. Mutational steps are one unless indicated by the numbers. Two haplotypes from TU more closely related to those from SL are exclusively found in males.

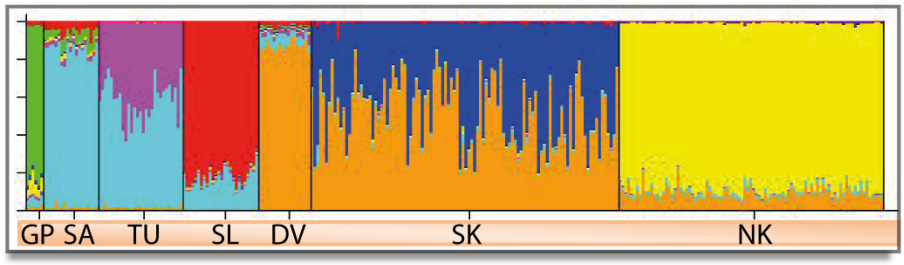


Microsatellite analyses

We also examined differentiation patterns using nuclear loci, which are biparentally inherited and therefore representative of both male and female histories, for the seven sites for which we could generate microsatellite genotypes. Both cluster analyses with *Structure* and significant pairwise population F_{ST} values indicate strong structuring of these sites (Fig. 2.2), particularly when separated by rivers (Fig. 2.1b). The structure runs for all seven sites using 12 microsatellite loci (Dataset II, Fig. 2.2a,) yielded the highest probability runs for K=7 (LnL -9619.88), partitioning each of the sites as a distinct cluster. Likewise, a more detailed analysis for the five sites for which 26 microsatellite loci were available (Dataset I) also led to each one being inferred as a separate cluster (SI, Fig. 2.3). Generally, high pairwise F_{ST} and level of structuring of populations is congruent with our mtDNA results. However, the cluster analyses using nuclear loci indicate some heterogeneity within populations. Since haplotype sharing is rare among populations exchanging migrants, the low levels of gene flow are most likely male-mediated.

Fig. 2.2 Population structure based on 12 nuclear microsatellite markers. (A) Structure run for the seven study sites with 12 microsatellite marker data (Dataset II) at K=7 (LnL -9576.8). (B) Table 1. Inter-population differentiation with pairwise FST estimates above the diagonal and pairwise ΦST estimates below the diagonal. All are significant at p<0.001, except when indicated: * p<0.05; ** p<0.01.

A



B

	DS	KU	GP	SA	SL	TU	DV	SK	NK
DS		-	-	-	-	-	-	-	-
KU	0.790*		-	-	-	-	-	-	-
GP	0.962	0.986		0.143**	0.160	0.163	0.209**	0.131	0.125
SA	0.880	0.882	0.159		0.110	0.049	0.152	0.083	0.069
SL	0.881	0.874	0.934	0.895		0.084	0.190	0.109	0.121
TU	0.758	0.680	0.827	0.801	0.779		0.117	0.072	0.090
DV	0.910	0.914	0.970	0.893	0.886	0.658		0.036	0.112
SK	0.812	0.754	0.845	0.810	0.814	0.628	0.163**		0.040
NK	0.879	0.854	0.896	0.859	0.879	0.748	0.689	0.409	

We investigated the signature of sex-specific demographic processes more directly by comparing isolation by distance patterns for the nuclear and mtDNA loci. The Mantel test for the relationship between genetic and Euclidean geographical distance yielded a significant and positive correlation for both the nuclear markers and mtDNA (F_{ST}: r=0.415, p<0.05; Φ_{ST}: r=0.357, p<0.05). We also explored the effect of rivers in a partial Mantel test of the association between genetic and cost path distances whilst controlling for Euclidean distance. Results were significant for the mtDNA (p<0.01; r=0.403) but not the nuclear markers (p=0.633; r=-0.096). It is noteworthy, however, that for the mtDNA, only three out of the 36 population pairs studied have low Φ_{ST} values (< 0.6). Therefore, most populations are highly differentiated from each other despite the short geographical distances between them.

2.5 Discussion

We investigated the evolutionary history of Bornean orangutans using the most comprehensive Bornean sample set compiled to date. Our mtDNA results indicate a surprisingly recent origin for current Bornean populations, and together with the nuclear markers, illustrate that their current distribution has been uniquely shaped by a combination of historical, geographical and socio-behavioral factors.

Historical factors: recent radiation of Bornean populations

The recent coalescence of Bornean orangutan haplotypes in the Middle to Late Pleistocene is in striking contrast with that of the other Bornean canopy-bound rainforest species for which data are available, the gibbon *Hylobates muelleri.* This gibbon, distributed throughout the north, east and west of Kalimantan, has a time to the most recent common ancestor (TMRCA) of 1.78 ma (1.33-2.25 95% CI) (Thinh *et al.* 2010), suggesting that Bornean gibbons have been differentiating within the island for much longer than orangutans. Moreover, Sulawesi macaques (genus: *Macaca*), which originated from the Bornean pig-tailed macaques, coalesce with them ~2 ma (Ziegler *et al.* 2007). Although the exact timing of their migration is uncertain, the older mtDNA coalescence dates for both Bornean gibbons and Bornean and Sulawesi macaques suggests they have been in Borneo as far back as the Early Pleistocene. Therefore it is conceivable that orangutans also arrived in Borneo around the same time. Yet, current Bornean orangutan mtDNA haplotypes stem from a very recent common ancestor originating in the Middle to Late Pleistocene.

The relatively short time to the most recent common ancestor of Bornean haplotypes is particularly striking given the deep Bornean-Sumatran orangutan coalescence approximately 3.5 ma. Such a long differentiation between Bornean and Sumatran haplotypes appears hard to reconcile with the recent episodes of interconnectedness between the islands during the Pleistocene glaciations, most notably during the Last Glacial Maximum (LGM) approximately 17 ka (Voris 2000; Bird *et al.* 2005). However, the presence of land bridges does not necessarily imply suitable conditions for migration. A savannah corridor (Heaney 1991) combined with riverine barriers dissecting the exposed land (Voris 2000; Harrison *et al.* 2006) would have presented severe obstacles to migration for orangutans, restricting them to riverine forest galleries along the banks. Coalescence for Bornean and Sumatran haplotypes is expected to vary across species, reflecting differences in dispersal abilities, habitat requirements, or ancestral effective population size, aside from possible discrepancies in dating methods (Pulquério &

Nichols 2007). For instance, the south Bornean gibbon *Hylobates albibarbis* and the Sumatran-Malaysian gibbon *Hylobates agilis* have a TMRCA of 1.56 ma (Thinh *et al.* 2010), and Bornean and Sumatran pig-tailed macaques have one of 3-4 ma (Ziegler *et al.* 2007). By contrast, the Bornean-Sumatran common ancestor of both the silvered langur (Roos *et al.* 2008) and clouded leopards (Wilting *et al.* 2007) is much more recent than that for orangutans, gibbons and pig-tailed macaques, probably due to a higher flexibility of habitat use.

Assuming that orangutans arrived in Borneo around the same time as gibbons and macaques, the recent coalescence of Bornean orangutans could be explained by a bottleneck through a severe rainforest contraction. Such a bottleneck would have had a more dramatic impact on the mtDNA structure of orangutans compared to other species, due to their low densities and slow life histories (Delgado & Van Schaik 2000) as well as habitat requirements. Gibbons were apparently not affected by habitat changes as harshly perhaps because populations can survive in smaller patches. Our findings are consistent with the survival and expansion of a single lineage from within a refugium in Borneo. Geomorphological and palynological data indicate the presence of dryer, more open vegetation in southern and western Borneo during the last glaciation (Meijaard 2004; Bird *et al.* 2005), and by extrapolation also during other glaciations (but c.f. Cannon *et al.* 2009; Woodruff 2010). Climate change was especially severe during an extended cold period within the penultimate glaciation between 130-190 ka (Martinson *et al.* 1987; Wright 2000), which occurred around the time of mean coalescence of Bornean mtDNA haplotypes. More recently, the last Toba eruption approximately 74 ka resulted in a short albeit significant decline in regional temperatures, ensued by a 1,800-year cold stadial (Rampino & Self 1992; Williams *et al.* 2009). Our data do not provide clear signals to make conclusive statements about potential Toba effects. Nonetheless, the coldest period of the penultimate glaciation (Martinson *et al.* 1987; Wright 2000) was more prolonged than the cold period following the last Toba eruption, suggesting more severe effects of the former on the extent of rainforest across Sundaland. In any event, suitable rainforest habitat for orangutans should have existed in certain regions in Borneo where a refugium population survived the dry glacial conditions. Possible Pleistocene refugia in Borneo have also been described for numerous other rainforest species such as termites, ants, orchids, oaks, and large-bodied mammals (Barkman & Simpson 2001; Gathorne-Hardy *et al.* 2002; Cannon & Manos 2003; Meijaard & Groves 2004; Louys 2007; Quek *et al.* 2007; Ziegler *et al.* 2007), and together with the isolation of the island, could act as a mechanism of evolutionary diversification driving high Bornean species endemism. Following the expansion of orangutans throughout the island, the Pleistocene climatic oscillations should have led to recurrent population expansions and contractions.

Geographical and socio-behavioral barriers

Despite the recent common ancestry of Bornean populations, our analyses revealed high and significant mitochondrial differentiation, with populations within currently recognized subspecies generally displaying as much differentiation as those between subspecies. Of notable interest is the great extent of subdivision and lack of reciprocal monophyly for the morphologically recognized subspecies *P.p.morio* and *P.p.wurmbii*. MtDNA haplotype sharing is uncommon and for populations separated by rivers occurs only in two instances: (i) for Sabangau and Gunung Palung and (ii) for the northern and southern populations across the Kinabatangan river. In both cases, very recent common ancestry could explain the incomplete mtDNA lineage sorting. For North and South

Kinabatangan, Jalil et al. (Jalil *et al.* 2008) proposed an expansion from a recent common refugium further west in Mount Kinabalu, as posited for other Bornean species (Barkman & Simpson 2001; Gathorne-Hardy *et al.* 2002; Cannon & Manos 2003). Danum Valley, with its low haplotype diversity, might also be the result of a recent range expansion. Gunung Palung is located proximally to the Bangka-Bilitung-Karimata-Schwaner divide, from where orangutans are presumed to have dispersed to the rest of Borneo (Rijksen & Meijaard 1999) and where we might expect a rich haplotype diversity. However, the presence of only one mtDNA haplotype shared with populations further east suggests that the current population in Gunung Palung is recent and/or underwent a severe recent bottleneck. This and other local bottlenecks make it impossible to reconstruct a colonization of Borneo through the southwestern "choke point"(Handley *et al.* 2007).

The rarity of mtDNA haplotype sharing among Bornean populations contrasts with patterns in the patrilocal chimpanzees and bonobos (Eriksson *et al.* 2006; Langergraber *et al.* 2007), where mtDNA sharing is extensive. Interestingly, two orangutan haplotypes from one site (TU) that were more closely related to those of another site (SL) pertain only to males. While nuclear differentiation among orangutan populations is significant, we find evidence for a small degree of nuclear gene flow, suggesting that it is male-mediated. Furthermore, the effect of rivers on the isolation by distance patterns for the mtDNA indicate that these are important barriers to female movement, probably as a result of smaller dispersal distances of females (Delgado & Van Schaik 2000). An association between mtDNA genetic distance and distances around rivers have also been found in gorillas (Anthony *et al.* 2007), and a role for differential dispersal distances between the sexes has been posited for western lowland gorillas (Douadi *et al.* 2007). Our results are consistent with the pattern of female philopatry and male-biased dispersal proposed by Delgado & van Schaik (2000) and indicate that the orangutan sexes are subject to very different constraints on mobility. While female philopatric behavior may be responsible for the strong effect of geographical barriers on mtDNA structure, we cannot make any conclusive statements on the effects of rivers on males. More continuous sampling especially along rivers and examination of Y-chromosomal markers, representative of male histories, will prove useful in determining how geographical barriers differentially affect the sexes. In addition, further geomorphological data on river course and width changes through time would contribute to the understanding of their vicariant action.

Bornean orangutan distribution and population structure has been uniquely shaped by the Pleistocene fluctuations and by socio-behavioral and geographical barriers to movement. Our findings support a recent radiation of Bornean orangutans in the Middle to Late Pleistocene, resulting in "static" clusters of females strongly separated by geographical barriers and subject to high differentiation, with more mobile males exerting a homogenizing influence on the nuclear gene pool. Further sampling will help establish whether there is a marker specific pattern of clusters versus clines resulting from sex-biased dispersal (c.f. Handley *et al.* 2007). In addition, in depth population genetic studies of other endangered and endemic taxa such as the Bornean gibbons and Sumatran orangutans will be of interest in contrasting the differential effects of environmental processes.

2.6 Acknowledgments

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2.8 Supporting Information

Materials and methods

DNA extraction and quantification

We extracted DNA using the QIAamp DNA Stool Mini Kit (QIAGEN) following the manufacturer's protocol with one modification: samples were allowed to incubate for a minimum of 30 minutes before elution. We quantified DNA through real-time quantitative polymerase chain reaction (rtPCR) using the protocol from a previous study (Morin *et al.* 2001b). The rtPCR assay allows determination of the number of positive PCR replicates per extract necessary to obtain a 99% confidence level that a homozygous genotype is correct (Morin *et al.* 2001b). For a heterozygous genotype, our criterion was the observation of each of the two alleles at least twice in independent PCRs.

MtDNA analyses

We sequenced a 323 bp region of the mitochondrial DNA (mtDNA) hypervariable region I (HVRI) using the primers DLF (5'-CCT GCC CCT GTA GTA CAA ATA AGT A-3') and D5 (Warren *et al.* 2001). PCR amplifications were performed in a 20 µL reaction volume containing 0.25 µM of each primer, 0.2 mM dNTPs, 1 x PCR Buffer (Qiagen), 2µ l Bovine Serum Albumin (BSA), 0.5 units HotStarTaq DNA Polymerase (Qiagen) and 1 µL template DNA. PCR conditions were as follows: initial denaturation at 95°C for 15 minutes, followed by 45 cycles of 94°C for 40s, 52°C for 30s, 72°C for 30s, and final extension at 72°C for 10 mins. Reactions were purified with the QIAquick PCR Purification Kit (Qiagen) following the manufacturer's recommendations. Cycle sequencing was performed in a 10 µL reaction volume containing 1 µL of purified PCR product, 1x sequencing buffer (80 mM Tris, 2 mM $MgCl_2$, pH 9.0), 0.4 µM forward primer and 0.3 µl BigDye Terminator v3.1. The cycle sequencing conditions were initial denaturation at 95°C for 45 seconds, followed by 30 cycles of 95°C for 30s, 52°C for 20s, 60°C for 2 mins. Capillary electrophoresis was carried out using the 3730 DNA Analyzer (Applied Biosystems).

All raw data were viewed and edited in Sequencing Analysis 5.2 (Applied Biosystems). The sequences were aligned with ClustalW (Thompson *et al.* 1994) in Bioedit 7.0.9.0 (Hall 1999) and collapsed using DAMBE 5.0.7.2 (Xia & Xie 2001).

Microsatellite analyses

To standardize the data sets from NK and SK, we genotyped at least 10 original extracts from NK and SK for each of the 12 loci. We ran these samples on the same instruments and analysed them in the same way as for the other study sites. In cases where NK and SK genotypes were found to differ due to bin set discrepancies, we adjusted allele sizes to match our current bin set. We were not able to complete genotypes for all 25 markers due to low DNA quantity and quality, probably due to long-term storage degradation.

All samples from all sites were subjected to identity analyses on Cervus 3.0 (Marshall *et al.* 1998; Kalinowski *et al.* 2007). If identical genotypes were found, only one was included in our data set.

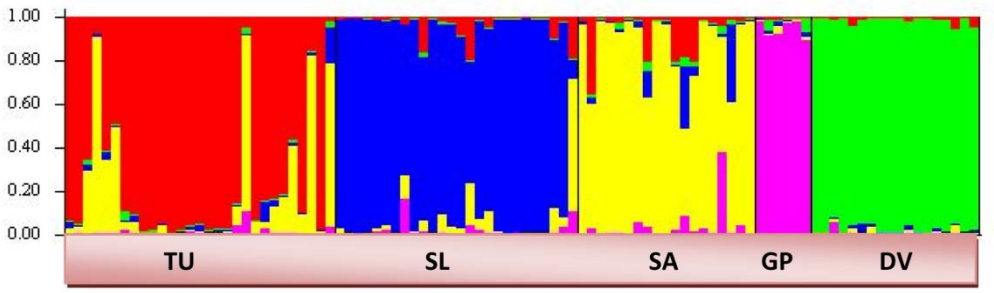
PCR amplifications were performed as multiplex reactions in an 8 µL volume containing 1 µL DNA, 4 µL Multiplex Master Mix (QIAGEN), 0.8 µL primer mix, and 2.2 µL water. Amplification conditions were: initial denaturation at 95°C for 15 minutes, followed by 40 cycles of 94°C for 30s, 58°C for 90s, 72°C for 1 min, and a final extension at 60°C for 30 mins.

We performed capillary electrophoresis on the 3730xl DNA Analyzer (Applied Biosystems). Products were analysed using GeneMapper v4.0 (Applied Biosystems).

We used Arlequin 3.11 to calculate deviation from Hardy Weinberg equilibrium (HWE) and GenePop 4.0 (Raymond & Rousset 1995; Rousset 2008a) to assess linkage disequilibrium (LD). We checked for allelic dropout and null alleles using ML-NullFreq (Kalinowski & Taper 2006b).

Figures

Fig. 2.3 Structure run for the five study sites with 25 microsatellite marker data (Dataset I) at K=5 (LnL -5395.4).



Tables

Table 2.1 Population average pairwise differences calculated with Arlequin 3.11. i) Above diagonal: average number of inter-population pairwise differences; ii) Diagonal elements: average number of intra-population pairwise differences; iii) below diagonal: Corrected average inter-population pairwise differences, computed using Tamura-Nei, gamma distribution with shape parameter 0.344.

Site	GP	SA	SL	TU	DS	KU	DV	SK	NK
GP	0	0.86	13.41	12.24	11.81	10.54	8.72	8.95	9.89
SA	0.15	1.42	14.27	13.23	12.11	11.54	9.64	9.91	10.99
SL	12.63	12.78	1.56	11.96	12.99	11.82	10.01	10.33	13.35
TU	10.44	10.72	9.38	3.6	11.28	9.73	6.85	7.61	10.14
DS	13.57	14.57	15.53	14.84	3.51	10.87	11.7	12.58	15.34
KU	10.02	10.31	10.52	7.42	8.6	1.04	7.34	8.17	10.72
DV	8.44	8.66	8.96	4.78	9.67	6.55	0.54	1.71	3.93
SK	7.86	8.11	8.46	4.72	9.73	6.56	0.35	2.18	3.23
NK	9.07	9.47	11.75	7.52	12.77	9.39	2.84	1.33	1.64

Table 2.2 Samples from the Warren et al. (2001) dataset included in analyses after re-sequencing. Table modified from Warren et al. (2001).

Code	Status[1]	Origin/Reference[2]	Site assigned
OU TNK41	W	Kutai National Park, EK	Kutai (KU)
OU TNK39	W	Kutai National Park, EK	Kutai (KU)
OU TNK37	W	Kutai National Park, EK	Kutai (KU)
OU TNK36	W	Kutai National Park, EK	Kutai (KU)
OU TP14	R	Tanjung Puting, CK	none
OU TP6	W	Tanjung Puting, CK	none
OU TP24	W	Tanjung Puting, CK	none
OU DSRA	W	Danau Sentarum, NK	Danau Sentarum (DS)
OU DSLE1	W	Danau Sentarum, NK	Danau Sentarum (DS)
OU DSME1	W	Danau Sentarum, NK	Danau Sentarum (DS)
OU DSME2	W	Danau Sentarum, NK	Danau Sentarum (DS)
OU SEUA	R	Semongok, Sarawak	None
OU SEBU	R	Semongok, Sarawak	None
OU SEOA	R	Semongok, Sarawak	None
OU SE8	R	Semongok, Sarawak	None
OU KPC	W	Sangatta, EK	None
OU KAI	W	Sangatta, EK	None
OU SB71	W	Sandakan, Sabah	None
OU SB60	W	Kinabatangan, Sabah	None
OU SB57	W	Sukau, Kinabatangan, Sabah	None
OU SB372	R	Sepilok, Sabah	None

[1] W = wild; R = rehabilitation centre

[2] Abbreviations EK = East Kalimantan; CK = Central Kalimantan; SWK = South West Kalimantan; NK = North Kalimantan

Table 2.3 mtDNA and nuclear microsatellite datasets.

Marker	Analyses	Samples included
mtDNA	Phylogenetic reconstruction	Resequenced Warren dataset and GP, SA, SL, TU, DV, SK, and NK
mtDNA	Population structure	Resequenced Warren sites KU and DS, and GP, SA, SL, TU, and DV
Nuclear microsatellites	Population structure	GP, SA, SL, TU, and DV (26 microsatellite loci)
Nuclear microsatellites	Population structure	GP, SA, SL, TU, DV, SK, and NK (12 microsatellite loci)

Table 2.4 Nuclear microsatellite loci amplified in the study.

Locus name	Sequence (5'-3')	Repeat	Reference
D1S550	F: CCTGTTGCCACCTACAAAAG	Tetranucleotide	(1)
D1S550	R: TAAGTTAGTTCAAATTCATCAGTGC	Tetranucleotide	(1)
D2S1326	F: AGACAGTCAAGAATAACTGCCC	Tetranucleotide	(1)
D2S1326	R: CTGTGGCTCAAAAGCTGAAT	Tetranucleotide	(1)
D3S2459	F: CTGGTTTGGGTCTGTTATGG	Tetranucleotide	(1)
D3S2459	R: AGGGACTTAGAAAGATAGCAGG	Tetranucleotide	(1)
D4S1627	F: AGCATTAGCATTTGTCCTGG	Tetranucleotide	(1)
D4S1627	R: GACTAACCTGACTCCCCCTC	Tetranucleotide	(1)
D4S2408	F: AATAAACTTCAACTTCAATTCATCC	Tetranucleotide	(1)
D4S2408	R: AGGTAAAGGCTCTTCTTGGC	Tetranucleotide	(1)
D5S1470	F: CATGCACAGTGTGTTTACTGG	Tetranucleotide	(1)
D5S1470	R: TAGGATTTTACTATATTCCCCAGG	Tetranucleotide	(1)
D13S321	F: TACCAACATGTTCATTGTAGATAGA	Tetranucleotide	(1)
D13S321	R: CATACACCTGTGGACCCATC	Tetranucleotide	(1)
D13S765	F: TGTAACTTACTTCAAATGGCTCA	Tetranucleotide	(1)
D13S765	R: TTGAAACTTACAGACAGCTTGC	Tetranucleotide	(1)
D16S420	F: ATTTCCTGAGGTCTAAAGCACCC	Dinucleotide	(1)
D16S420	R: TTAGGCCCAGTCCACACTCAAG	Dinucleotide	(1)
D2S141	F: ACTAATTACTACCCNCACTCCC	Dinucleotide	(1)
D2S141	R: TTTTCCAAACAGATACAGTGAACTT	Dinucleotide	(1)
D5S1505	F: TAAGTGCCAGAGTCTCCCAC	Tetranucleotide	(1)
D5S1505	R: TAAGGCATGTCTCGGAGCTA	Tetranucleotide	(1)
D6S501	F: GCTGGAAACTGATAAGGGCT	Tetranucleotide	(1)
D6S501	R: GCCACCCTGGCTAAGTTACT	Tetranucleotide	(1)
D5S1457	F: TAGGTTCTGGGCATGTCTGT	Tetranucleotide	(1)
D5S1457	R: TGCTTGGCACACTTCAGG	Tetranucleotide	(1)
O4_6	F: GGCAATGTAACATATCCCTCTGTGT	Tetranucleotide	(2)
O4_6	R: AGCCATGGACCTTGTGAGAAAAG	Tetranucleotide	(2)
O4_A5	F: ATGGGCCCAGAAAACAACTCAGT	Tetranucleotide	(2)
O4_A5	R: AGATAAAGGAATGGATAGATGGACAGA	Tetranucleotide	(2)
O4_B5	F: GAGCCCTGATTCGTTTTACTGG	Tetranucleotide	(2)
O4_B5	R: AGCAAAGGCAGAAAACTGTAATGA	Tetranucleotide	(2)
O4_B6	F: TGGAGCCTGAATATGTGACTGAAT	Tetranucleotide	(2)
O4_B6	R: AATGCCAGGATTTCCTTCTTTTT	Tetranucleotide	(2)
O4_A7	F: ACTGGCCCATTCAAAGTCTGTCATT	Tetranucleotide	(2)
O4_A7	R: ACTGGCCCATTCAAAGTCTGT	Tetranucleotide	(2)
O4_A1	F: CTCCCCTTCCTTCCTTTATTCAGTT	Tetranucleotide	(2)
O4_A1	R: CAACACTTGGCAGTCACAAATCAG	Tetranucleotide	(2)
O4_B17	F: GTACCGACGGTGCACGAACAATGTA	Tetranucleotide	(2)
O4_B17	R: AGCCTGGCTGAAAAGTGGAACTGAG	Tetranucleotide	(2)
O4_B20	F: CCTGCATTTTGTCACTCCCTCAACC	Tetranucleotide	(2)
O4_B20	R: CTGCCACACCTCCATGGACACAGAT	Tetranucleotide	(2)
O4_B3	F: TTCCAGAAGGGGCGAGAAGTT	Tetranucleotide	(2)
O4_B3	R: GTTGGGACCAAACAGTTGTCAATAA	Tetranucleotide	(2)
O4_C13	F: CTGGGCACACTGTATATGGGGTAG	Tetranucleotide	(2)
O4_C13	R: GTTTGAGACCACTCATGATGCAAAGACC	Tetranucleotide	(2)
O4_C9	F: TGCAGGCCAGGGCTTCTTTCAA	Tetranucleotide	(2)
O4_C9	R: CAGTCTCCCCAGGACCCCTACACAG	Tetranucleotide	(2)
O4_Chr5	F: CAGCAGCTCCTGAAATATCTGTCC	Tetranucleotide	(2)
O4_Chr5	R: GTTTGGGGTAGAGGAAAGCAGGTTGAT	Tetranucleotide	(2)
O4_Chr7	F: CATCTCTTTATGGCTGACTGTTGAT	Tetranucleotide	(2)
O4_Chr7	R: GTTTGGTCCAAGACAAATTTGTATGAGT	Tetranucleotide	(2)

(1)(Goossens *et al.* 2005), (2) (Nietlisbach *et al.* 2010)

2.9 Addendum: Population size changes

While highly differentiated for their mtDNA, Bornean populations are monophyletic and share a relatively recent common ancestor. Our analyses show that an ancestral population is likely to have expanded spatially and demographically in the past. We have further investigated expansions both in the ancestral population and in current populations by conducting test statistics on site frequency spectra.

Such test statistics can be used to detect selection or in the case of neutrally evolving markers, they can be used to uncover population size changes. We computed the following site statistics, also referred to as neutrality tests, using the mtDNA control region HVRI sequences, assumed to be under neutral evolution: Tajima's D (Tajima 1989), Fu's F_S (Fu 1997), and Fu and Li's D* (Fu & Li 1993) (see Appendix I for further details). A population expansion is expected to result in an excess of rare singleton variants. This change in the allele frequencies of a growing population compared to a constant population is reflected in negative values of these tests (Tajima 1989; Fu & Li 1993; Fu 1997). Of these tests, Fu's F_S is reported to be the most sensitive measure to population expansion (Fu 1997; Ramos-Onsins & Rozas 2002). First, we carried out the tests only on the resequenced samples from Warren et al. (2001) which contained a lower number of polymorphisms than the published dataset. Next, we carried out the tests using not only the resequenced Warren dataset but also the population data (GP, SA, SL, TU, DV, SK, and NK) thus encompassing a wider area of the distribution of the Bornean orangutans. The tests were carried out using DNAsp and Arlequin and implementing 10,000 bootstrap replicates to determine significance levels.

Our results show a trend for Fu's F_S for Warren et al.'s (2001) dataset alone (n=24), and significant values when combined with the present study's population based data (n=221) (Table 2.5). The results suggest a Bornean wide expansion. It is plausible, nevertheless, that recent contraction events owing to human-induced habitat fragmentation confounds the signal. Two previous studies (Steiper 2006; Jalil *et al.* 2008) based on published sequences from Warren et al. (2001) also reported signals of Bornean wide expansions detected by Fu's F_S. While Steiper (2006) used Warren's dataset alone, Jalil et al. (2008) combined it with population based data from the Lower Kinabatangan Wildlife Sanctuary. The published dataset from Warren et al. (2001) they used, however, appears to contain many more polymorphisms compared to the dataset used herein, which could explain the discrepant levels of significance.

Table 2.5 Neutrality tests for all of Borneo.

Group	n	Tajima's D	Fu's F_s	Fu and Li's D*
Resequenced Warren	24	-0.135	-3.833~	-0.728
Resequenced Warren + Pops [1]	221	-0.140	-9.432*	0.170
Steiper, 2006 [2]	36	-1.56	-28.42***	-2.38
Jalil et al., 2008 [3]	102	-1.582*	-18.897***	-3.140*

~ = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

[1] Populations from the present study as well as the resequenced data from Warren et al. (2001).

[2] Published dataset from Warren et al. (2001)

[3] Published dataset from Warren et al. as well as the data for the Lower Kinabatangan Wildlife sanctuary (LKWS) generated by Jalil et al. (2008)

There are a few issues associated with these tests, however, which might explain why not all of them reveal clear signals for population size change. One of the key assumptions of the tests is that of panmixia. Detecting expansions in subdivided populations with low levels of migration, the case for Bornean orangutan populations, is therefore problematic (Ray *et al.* 2003; Stadler *et al.* 2009). Recent studies indicate that random sampling may emulate genealogical properties of panmictic populations (De & Durrett 2007). In fact, the most powerful sampling scheme in recovering true expansions under both a stepping-stone and island model is that of scattered sampling, which implies using single sequences from each population (Stadler *et al.* 2009). Another issue is that of selection instead of drift driving the results of these test statistics, for instance, if the sequences used are subject to hitchhiking selection. The low likelihood of this possibility is addressed in detail in Chapter 5, the general discussion.

Test statistics for each separate population did not detect population size changes (Table 2.6), as none of the test statistics were significant. However, further analyses on expansions and contractions are being conducted using the nuclear microsatellite data generated through our collaboration with other researchers. Preliminary results point to extremely recent population contractions within the last millennium at most of the individual sites (Sharma 2011).

Table 2.6 Neutrality tests per population.

Population	Tajima's D	Fu's F_s	Fu and Li's D*	Fu and Li's F*
Pop 1 - TU	-1.081	0.373	0.350	-0.119
Pop 2 - SL	0.360	2.592	1.158	1.075
Pop 3 - SA	-0.363	-1.797	0.448	0.241
Pop 4 - DV	1.548	1.428	0.667	1.024
Pop 5 - NK (various)	0.162	-1.920	-0.401	-0.268
Pop 6 - SK (various)	-0.157	-0.811	0.124	0.041

3 Genetic and behavioral evidence for stacked matrilineal female clusters and male-biased dispersal in a population of Bornean orangutans (*Pongo pygmaeus*)

3.1 Abstract

Philopatry and sex-biased dispersal have a strong influence on population genetic structure, so the study of species dispersal patterns and evolutionary mechanisms shaping them are of great interest. Particularly non-gregarious mammalian species present an underexplored yet exciting field of study: despite their lower levels of sociality compared to group-living species, interactions among individuals do occur, providing opportunities for cryptic kin selection. Among the most enigmatic non-gregarious species are orangutans, in which preferential associations among females have been observed, but for which the presence of kin structures is unresolved due to the equivocal results of previous genetic studies. With a view to clarifying relatedness and dispersal patterns in orangutans, we examined a site with the largest set of genetically characterized individuals (n=41) to date for which behavioral and spatial data was also available. Using a suite of markers including the mitochondrial DNA (mtDNA) hypervariable region I (HVRI) and 24 biparentally inherited microsatellite markers, we tested a set of qualitative predictions for four different dispersal models. The expectations for female philopatry and male-biased dispersal include more close maternal relatives among females compared to males, and greater mtDNA diversity levels of males reflecting more varied maternal ancestries. In agreement with these predictions, our pedigree reconstruction demonstrated the presence of three matrilineal clusters of females generally comprising closely related pairs that overlapped in space use. In general, no mothers or maternally related siblings were assigned to the males, with the exception of one young individual. Males displayed high mtDNA variation. Furthermore, while one mtDNA haplotype was shared with peripherally ranging females, most male haplotypes were unique, underscoring their different maternal ancestries compared to females. These findings demonstrate that orangutan females are characterized by extreme site fidelity, whereas males disperse whenever they can. We discuss several factors that affect the sensitivity of genetic measures to detect sex-biased dispersal in non-gregarious species, and the new avenues of research opened by our findings to investigate a role for cryptic kin selection.

3.2 Introduction

Natal sex-biased dispersal, whereby one sex displays a greater tendency to leave or travel longer distances away from the natal area to breed, is ubiquitous in the animal kingdom (Howard 1960; Clobert *et al.* 2001). This crucial life history trait has a strong impact on population genetic structure, influencing the maintenance and loss of genetic diversity in populations (Chesser 1991; Sugg *et al.* 1996; Storz 1999). Hence, resolving patterns of dispersal in a species, and the mechanisms underlying these are of great interest.

Some of the evolutionary mechanisms invoked to explain the tendency for one sex to exhibit site fidelity or philopatry, that is, the tendency to breed within or in close

proximity to the natal range, include ecological benefits. For instance, philopatric individuals might benefit from familiarity with resources and avoid the risks associated with migration through unknown areas (Greenwood 1980; Lawson Handley & Perrin 2007). Philopatry results in kin structures that might also confer benefits of another nature, those of nepotistic interactions, providing inclusive fitness benefits that could augment or even drive philopatry (Perrin & Goudet 2001; Lawson Handley & Perrin 2007). The prediction for species with mate-defense mating systems as prevalent among mammals, are that females, who benefit most from acquaintance with territory, should show philopatry, with males dispersing to avoid local mate competition and inbreeding (Greenwood 1980; Dobson 1982; Pusey 1987; Wolff 1993). And indeed, field and genetic studies have largely confirmed the expectation, although not to be neglected are the puzzling exceptions to the "phylogenetic inertia" of mammalian social organization (Pusey 1987; Favre *et al.* 1997; Hammond *et al.* 2006; for a review see Lawson Handley & Perrin 2007).

In group living mammals, the abundance of social interactions, together with the possibility to distinguish social units, has prompted many genetic studies to determine whether kin structure underlies social behaviors such as tolerance, cooperation, learning, and cultural variation (spotted hyenas; Van Horn *et al.* 2004; chimpanzees, Lukas *et al.* 2005; horses; Cameron *et al.* 2009; chacma baboons, King *et al.* 2011). Few studies, however, have examined relatedness patterns in non-gregarious species due to their lower levels of sociality, and the absence of recognizable cohesive units, which draws less interest and makes them more difficult to examine. Nevertheless, individuals of non-gregarious species do have "social networks" (Richard 1985), engaging in associations with neighbors, so there are opportunities for cryptic kin selection to operate (Hatchwell 2010). Consequently, the exploration of kin structures in such species may lead to exciting new insights.

The few genetic investigations to date of non-gregarious mammals have concentrated on carnivores (raccoons; Ratnayeke *et al.* 2002; cougars; Biek *et al.* 2006; bears; Zedrosser *et al.* 2007) and rodents (woodrats; McEachern *et al.* 2007 van Staaden et al., 1994), as well as a few lemur species among the primates (Kappeler *et al.* 2002; Eberle & Kappeler 2006; Radespiel *et al.* 2009). Such studies have proven invaluable, as illustrated by the examination of the solitarily foraging grey mouse lemur, a species in which females allo-nurse in diurnal sleeping groups. The usage of genetic markers enabled Eberle & Kappeler (2006) to establish that allo-nursing females comprised close maternal relatives, thus providing strong evidence for kin cooperative breeding. While cooperative breeding is an overt form of cooperation, it is noteworthy that kin selection might also act through less obvious ways, for instance, through reduced aggression and increased tolerance towards relatives that might make settlement in familiar areas easier, or through inheritance of high matrilineal social status (Perrin & Goudet 2001; Hatchwell 2010).

Undoubtedly, among the non-gregarious species one of the greatest enigmas presented is that of the orangutans, the Asian great apes. Although most apes show fission-fusion sociality, orangutans are at the solitary end of this continuum (van Schaik 1999). Nonetheless, orangutans do interact with each other in travel parties, in aggregations at fruit trees, and during encounters that provide opportunities for play among infants (van Schaik 1999; Singleton & van Schaik 2002; Van Noordwijk 2011b). Interestingly, orangutans also show striking preferential association as demonstrated by Singleton and van Schaik (2002) in their study of a Sumatran populations. The authors found evidence

for higher likelihood of association among females of a cluster, who had widely overlapping home ranges, exhibiting reproductive synchrony, and showed high morphological resemblance suggestive of relatedness.

Nevertheless, the relatedness among the females of such clusters has not been genetically confirmed, which is important because while behavioral studies point to female philopatry and male-biased dispersal , individual identification is often difficult and the movement of individuals does not necessarily imply reproduction (Prugnolle & de Meeus 2002). In fact, the general patterns of relatedness among females and males in orangutan populations are not clear due to the equivocal result of genetic studies of historical and contemporary gene flow. Studies of historical dispersal have revealed greater geographical structuring and differentiation of mtDNA markers compared to Y-chromosome or biparentally inherited markers. Hence, these studies support historical female philopatry and male-biased dispersal (Chapter 2; Arora *et al.* 2010; Nater *et al.* 2011a; Nietlisbach & Kruetzen 2011). By contrast, the three investigations of current dispersal patterns at different sites have all produced varying results. These one-generation dispersal studies were based on the conventional genetic method that relies on the comparison of average pairwise relatedness (r) estimates of adult females and adult males obtained using biparentally inherited microsatellite markers. The expectation is that the more philopatric sex comprising related individuals should have higher r values than the dispersing sex comprising immigrants (Prugnolle & de Meeus 2002; Lawson Handley & Perrin 2007). The first study, at the Sumatran site of Ketambe, found similarly low r coefficients for females and males, suggesting that they both disperse yet the inclusion of rehabilitant females may have been a confounding factor (Utami *et al.* 2002). The second study, at the Bornean site of the Lower Kinabatangan Wildlife Sanctuary, showed similarly high r values in both sexes, indicating that females and males are both philopatric, yet in this case habitat fragmentation could have impeded the normal movement of individuals (Goossens *et al.* 2006b). Finally, the third study, which was conducted at a Bornean field site not affected by fragmentation or inclusion of rehabilitants, produced higher r values for the females compared to males, pointing to female philopatry and male-biased dispersal (Morrogh-Bernard *et al.* 2010). This recent study was, however, based on a smaller sample size than previous studies.

The discrepant behavioral and genetic results render the social organization of orangutans unresolved. It is also unclear whether contemporary dispersal patterns are at odds with historical patterns. Determining whether orangutans have kin structures and what their dispersal patterns are is necessary before we can investigate the possible evolutionary mechanisms that underlie these patterns, and which consequently affect the structuring of population genetic diversity. In addition, elucidating the social organization of these highly endangered species should be of use in conservation management programs. In a translocation program for instance it may be more problematic to mix individuals of the philopatric sex, if their movement results in fitness costs.

In order to gain an insight into the dispersal patterns of orangutans, we have drawn on the ongoing long-term study at Tuanan Orangutan Research Area, Borneo, Indonesia. We complemented behavioral and spatial data for the largest set of genetically characterized individuals (n=41) from a natural population of orangutans. Through the use of the maternally inherited mitochondrial DNA (mtDNA) as well as 24 autosomal microsatellite markers, we examined mtDNA diversity patterns, which are indicative of maternal co-ancestry, and carried out a parentage-based pedigree reconstruction to assess genealogical

relationships. Using this integrated approach, a set of qualitative predictions for the different dispersal models were tested (Fig.3.1). The predictions take into account the larger home ranges of orangutan males compared to females (Delgado *et al.* 2009), therefore referring to male philopatry as natal range expansion. The predictions also focused on maternal relatedness because in mammals philopatry is associated with staying at or in proximity to the maternal range. The models and predictions are outlined below:

A) Female philopatry and male-biased dispersal (MBD): females settle in the natal range, and males migrate away, so males are expected to comprise immigrants from "foreign" maternal lineages. The model predicts sex-specific mtDNA haplotypes, as well as higher mtDNA diversity of males compared to females. We also expect close maternal relatives among the females, and few or no relatives among the males (unless they engage in parallel dispersal, unlikely for behavioral reasons, see *Discussion*). Especially, mother-daughter pairs should be common, while mother-son pairs should be rare.

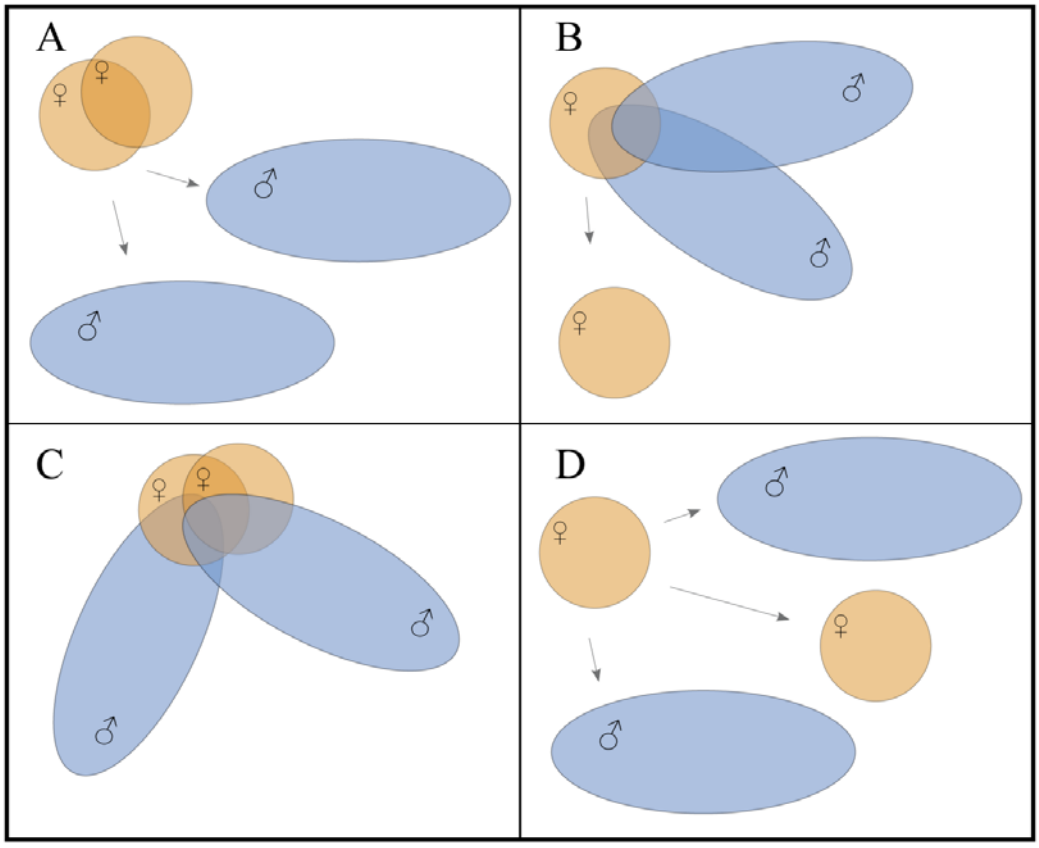
B) Male natal range expansion and female-biased dispersal (FBD): males remain in the natal area and females disperse. Males are thus expected to comprise close paternal relatives, while females should have few or no relatives. Hence, while father-son pairs should be common, mother-daughter pairs should not. No predictions are made concerning mtDNA diversity because it is maternally transmitted and both sexes might vary in their maternal ancestries to an uncertain extent.

C) Female philopatry and male natal range expansion: both sexes remain in the natal area. Males have larger home ranges than females but should occasionally be sampled in their natal areas. Hence, similar or slightly higher mtDNA diversity of males compared to females is expected. Importantly, we predict closely related dyads in both sexes, including the finding of mother-daughter and mother-son pairs.

D) Female and male dispersal: both sexes leave the natal area, thus the prediction is of similarly high levels of mtDNA diversity, and few close relatives among females or males except in the case of parallel dispersal. This latter case would nevertheless lead to an absence of mother-offspring pairs among the adults.

Our results provide strong support for the predictions for extreme female philopatry and male-biased dispersal, showing striking evidence for tight clusters of female relatives that overlap in their home ranges. We highlight some important characteristics of kin structures in orangutans which affect the power of tests to discern them, and discuss the significance of female philopatry for future studies.

Fig. 2 Schematic representation of dispersal models tested. Female home ranges are shown as orange circles, male home ranges as blue ellipses to illustrate their larger size. A family of a mother, daughter and two sons is shown. A) Female philopatry and male-biased dispersal: the daughter settles within the maternal range, and the two sons move away: B) Male natal range expansion and female dispersal: the two sons expand from the maternal range, the daughter moves away; C) Female philopatry and male natal range expansion: the daughter and two sons overlap in the maternal range; D) Female and male dispersal: the daughter and two sons move away from the natal range.



3.3 Materials and methods

Study site and behavioral information

Sampling was conducted in the Tuanan Orangutan Research Area (2.151° South; 114.374° East), Mawas Conservation Area, Central Kalimantan, Indonesia. This site is located within a peat-swamp forest and comprises more than 750 ha of grid-based trails. The orangutan density estimate for the area is approximately 4.25 ind/km^2. The females at this site have home ranges estimated at 250-300 ha (Wartmann et al. 2010). Males have home ranges far larger than those of females, but their precise sizes and the possible differences between the two male morphs, flanged and unflanged, are unknown. Behavioral data at this site has been collected since 2003 and includes information on space use, frequency of sightings, sex and age. The age of individuals born after 2003 was either known or estimated to the closest year; for individuals born before 2003, age was estimated based on known landmark ages in orangutans (see Wich *et al.* 2004).

Genetic dataset

An intensive sampling regime spanning from 2003-2009 allowed us to obtain non-invasively collected DNA samples for a total of 41 individuals unique individuals ($n_{females}$ = 19; n_{males} = 22), the largest dataset of behaviorally and genetically characterized individuals at any orangutan site to date. These numbers also correspond to the entire number of adult females encountered at the site, while for the adult males, there were only 6 additional individuals for which samples had not been available at the time of the study. We genotyped all individuals at 24 nuclear microsatellite markers that were in Hardy Weinberg Equilibrium (HWE), and showed no evidence of linkage disequilibrium (LD) or null alleles (NA). We also sequenced 450 bp of the hypervariable region I (HVRI) of the mtDNA. Genotyping and haplotyping protocols as well as HWE, LD and NA analyses are described in Chapter 2 (Arora *et al.* 2010). Our analyses included, unless specified otherwise, only adult individuals who have potentially already settled within the natal area or dispersed to breed so as to avoid biases (and Prugnolle & de Meeus 2002; see Lawson Handley & Perrin 2007). These "post-dispersal" individuals were sexually mature and regularly ranged independently from the mother (i.e. ranging at more than 50m distance for at least several consecutive days). The criteria resulted in a total of 32 post-dispersal individuals ($n_{females}$ = 15; n_{males} = 17).

Maternal lineages

Several analyses using the HVRI region were conducted to assess patterns of mtDNA diversity and maternal ancestry. First, individuals were assigned to maternal lineages based on their mtDNA haplotypes. Second, we compared levels of mtDNA nucleotide and haplotype diversity for females and males using DNAsp v.5.0 (Librado & Rozas 2009). To show the mutational distances between the haplotypes found in the population as well as their frequencies according to sex, we generated a median joining network using Network 4.0. For these analyses, an additional male was included due to its distinct mtDNA haplotype, although low sample DNA quality and quantity precluded a complete nuclear genotype.

Spatial distribution and observation frequency analyses

We also investigated the spatial distribution of mtDNA haplotypes using ArcGIS v.9.3.1 (ESRI 2008a). First, we plotted gravity points for each individual as the average of all locations at which it was observed. We manually checked that each plotted point was within or in close proximity to areas frequented by the individuals, and was not a potentially misleading artifact of averaging. Next, we used the HRT plug-in for ArcGIS (Rodgers *et al.* 2007) to calculate 95% kernel probability plots for the various maternal lineages, each of which comprised the aggregated spatial data for all females sharing a given mtDNA haplotype. For two of the matrilines, the home ranges of females were either fully or mainly within the study site. For a third matriline, however, ranging data of individual females were incomplete as they frequently moved outside of the study area. Nevertheless, for all maternal lineages, the matrilineal kernel probability plots faithfully represent areas within the study site where females were observed.

Parentage-based pedigree analyses

We examined the precise genetic relationships of female-female, male-male and female-male dyads through a combination of parentage and mtDNA analyses. First, a parentage analysis for all 41 individuals including infants and adolescents using the likelihood based approach was implemented in Cervus 3.0 (Kalinowski *et al.* 2007). Simulations were conducted in order to determine critical values of the log-likelihood score for a 95% confidence parentage assignment. The parameters for these simulations were 10,000 cycles and a minimum of 10 loci typed. The specified genotyping error rate of 0.112% was determined through the "repeat-genotyping" and "unintentionally resampled individuals" approaches described by Hoffman and Amos (2011a). Only sexually mature individuals old enough to have sired offspring were incorporated as candidate mothers or fathers, resulting in 30 candidate males and 13 candidate females. The proportion of candidate parents was estimated from field data, but given the large influence it has on the statistical significance of the results (Krützen *et al.* 2004), several values for this parameter (0.05, 0.08, and 0.10) were tested to check the robusticity of assignments. We further verified that the confidence level was suitable for further analyses by confirming all 7 mother-infant relationships known from field data. To be maternally related, dyads had to additionally fulfill the criterion of mtDNA haplotype sharing.

Next, a maternal sibship was inferred for individuals that shared a mother and a paternal sibship for individuals that shared a father. This parentage-based pedigree reconstruction allowed assessment of the number of maternal and paternal relatives at the site for each individual, incorporating parent-offspring and sibling relationships. We also examined the number of mother-offspring dyads as a percentage of the total number of female-female dyads and male-female dyads from each maternal lineage. These numbers represent only a minimum bound because the inference of genealogical relationships requires assignment to a parent, and hence, sampling of this parent, which might not be at the study site due to emigration or death.

3.4 Results

Maternal lineages

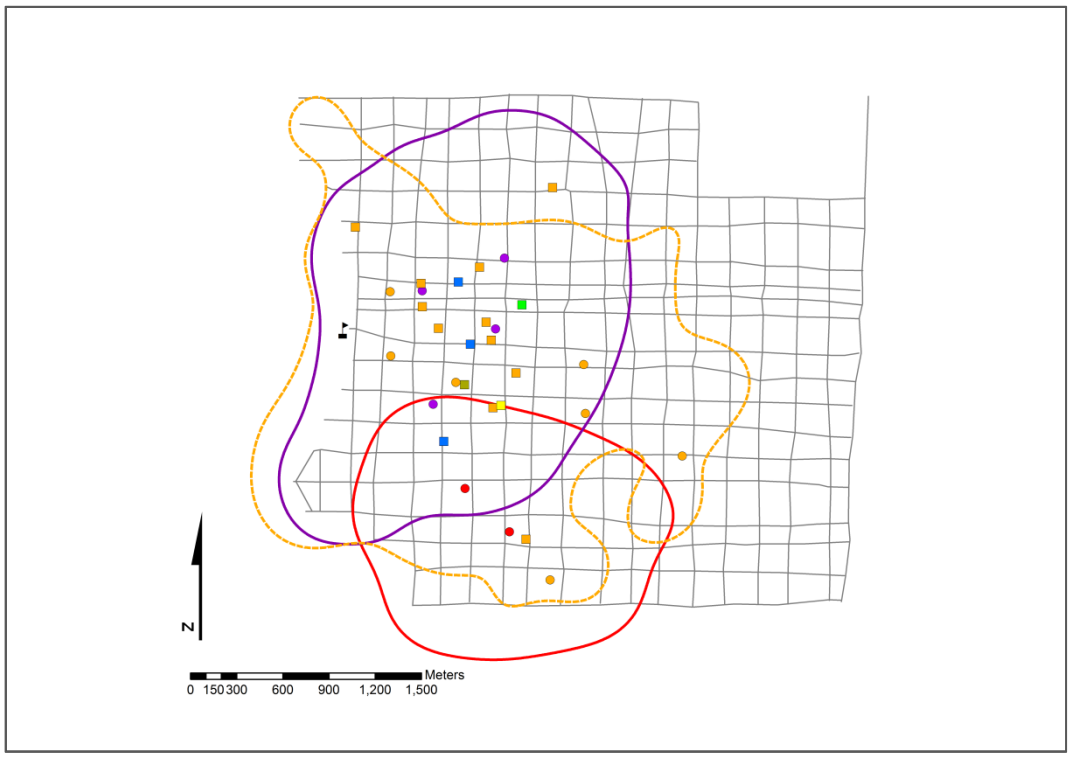
We investigated the maternal ancestry of individuals by examining maternal lineages and mtDNA diversity patterns. In total, we found eight different mtDNA haplotypes in Tuanan. The haplotype diversity and standard deviation was 0.621±0.091, and nucleotide diversity and standard deviation was 0.006±0.002. Two haplotypes were specific to females: haplotype B was found in 4 females (12% of individuals) haplotype B in 2 females (6%). Another haplotype (A) was very common, found in 20 individuals, and shared by both males (33%) and females (27%). The other five haplotypes were all male-specific, haplotype D was present in 3 males (9%) and haplotypes E, F, G, and H in one male each. Interestingly, two of the rare haplotypes unique to the males differ by at least 9 mutational steps from the other haplotypes (Fig. 3.2). The mtDNA variation led to a ten-fold higher mtDNA nucleotide diversity in males (π = 0.01±0.003) compared to females (π = 0.001±0.0003). Both the presence of sex-specific haplotypes and the high mtDNA diversity in males compared to females are consistent with the genetic predictions for female philopatry and male dispersal.

Fig. 3.2 MtDNA haplotypes in Tuanan. A median joining network of mtDNA haplotypes found in Tuanan is shown. Each colored circle represents a haplotype: hatched slices represent proportion of females with a particular haplotype; non-hatched slices represent proportion of males with that same haplotype. Number of mutations between haplotypes is one unless specified.

Spatial distribution analyses

The females at the study site belong to three different maternal lineages, which overlap extensively in ranges (Fig. 3.3). The females with haplotypes B and C had their home ranges fully or mainly with the core of the study area. However, the females with the most common mtDNA haplotype A frequently disappeared out of the study area, either to the north, east or south, illustrating their peripheral ranging. For the males, home range sizes are larger than the study area and thus unknown. We can, however, examine the frequency of their sighting according to their maternal lineage. This analysis revealed that 5 of the 7 males with rare haplotypes, which were not shared with the females, were observed frequently within the study area, having been sighted in over 30% of the months. By contrast, only 1 out of 11 males with the common A haplotype was observed equally or more frequently within the study site.

Fig. 3.3 Spatial distribution of mtDNA lineages and individuals in Tuanan. The grid represents the study site. Lines correspond to the combined ranges of females sharing a haplotype: orange dashed line (females with haplotype A, incomplete home ranges due to partial peripheral ranging), purple solid line (females with haplotype B, complete home ranges), red solid line (females with haplotype C, almost complete home ranges). Circles and squares represent the average of the spatial locations at which females and males were observed, respectively. Lineages and individuals are color-coded according to haplotype, following Fig. 3.1.



Parentage-based pedigree analyses

Through the reconstruction of a parentage-based pedigree, we were able to examine the genealogical relationships among individuals: we examined the distribution of maternal and paternal relatives as well as the distribution of mother-offspring dyads, among females and males (Table 3.1 and Fig. 3.4).

Our results showed that the females at the study site are part of three maternal lineages with distinct haplotypes (A, B, and C). These clusters contain close maternal relatives with generally high percentages of mother-daughter dyads (Fig. 3.4). Cluster A, comprising 9 females most of which range partly outside the study area, contained two mother-daughter pairs. One pair disappeared frequently to the north and the other to the east. Clusters B and C, comprising 4 and 2 females, respectively, with home ranges fully or mainly within the study site, consisted of sets of close relatives. In cluster B, we confirmed the presence of a mother and her three adult daughters, two of which in turn have adolescent female offspring. In cluster C we confirmed the presence of a mother and her adult daughter. One other female had her home range mainly within the study area, but belonged to cluster A, with no known relatives. Field observations indicate that this female had gradually moved from the disturbed habitat in which she had formerly ranged, and was consistently chased away at every encounter with females from the other

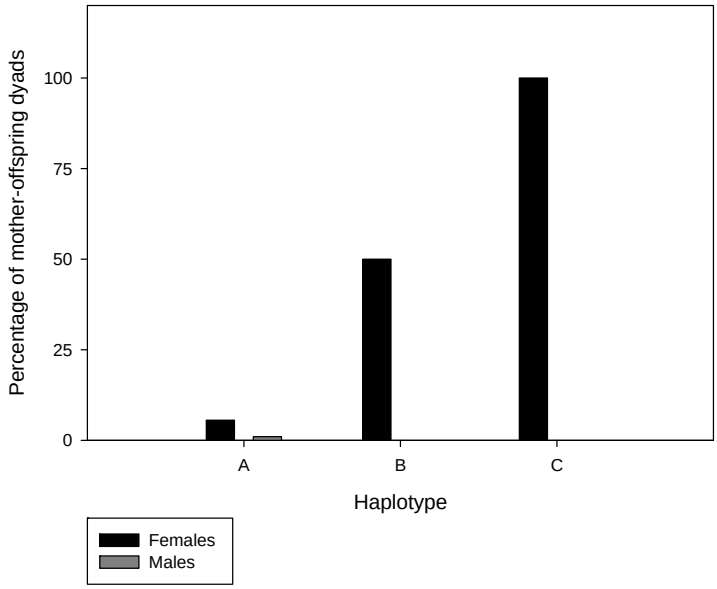
maternal lineages. No fathers were assigned to the females. In sum, among all 15 females, 10 had at least a mother or daughter at the site. Among the females whose home ranges are fully or mainly within the study site, all had a mother and/or daughter overlapping in range.

By contrast, and agreeing with our predictions for a model of male-biased dispersal, only one young male had a mother assigned to him (Fig. 3.4). The mother, as well as her adult daughter were both from cluster A, ranging only partly within the study site. None of the males shared haplotypes with the well-known females from clusters B and C whose home ranges were largely within the study site, indicating that this is not their natal area. No fathers or parental relatives were assigned to the males either.

Table 3.1 Maternal and paternal relatives of females and males at Tuanan. The number females and males with female maternal relatives and male paternal relatives at the study site is provided, with percentages in parentheses.

Sex	**No. individuals**	**With maternal relatives**		**With paternal relatives**	
		Females	**Males**	**Females**	**Males**
Females	15	10 (67%)	2 (13%)	0 (0%)	0 (0%)
Males	17	1 (5.88%)	0 (0%)	0 (0%)	0 (0%)

Fig. 3.4 Maternal relationships among females and males. The figure shows the percentage of mother-offspring dyads from the total number of female-female and male-female dyads sharing a maternal lineage.



3.5 Discussion

We integrated spatial, observational and genetic data to investigate the dispersal pattern in a non-gregarious mammal for which previous genetic studies produced mixed results. Drawing on the largest sample set to date from a natural population of orangutans, we tested predictions for different dispersal models through analyses of mtDNA diversity, and parentage-based pedigree relationships. We found sex-specific haplotypes and higher mtDNA diversity among males compared to females, underscoring the divergent maternal ancestries of the males. Importantly, our results showed the presence of close maternal relatives among the females, but a general absence of relatives among males, supporting a model of female philopatry and male-biased dispersal. We discuss these findings in detail and highlight the insights that emerged from this study on factors affecting our ability to disentangle dispersal patterns in non-gregarious species with slow life histories such as orangutans. Having determined a matrilineal kin structure in orangutans, we emphasize the interesting avenue open to research on the underpinning evolutionary mechanisms of dispersal in these species.

Female philopatry: evidence and comparisons

For the females, our findings showed that the different maternal lineages constitute clusters of close maternal relatives. The overlap in home ranges among these result in spatially stacked matrilineal clusters. Interestingly, for most of the females whose home ranges are encompassed within the study site, those with haplotypes B and C, we were able to fully disentangle maternal relationships. Although the relationships among females that range peripherally, those with haplotype A, are less complete, we were able to detect a mother-daughter pair that frequently disappeared to the north and another that frequently disappeared to the south.

Close maternal relatives among orangutan females support a model of female philopatry. But, while this fits the dispersal patterns found in some other “solitary” primates, some marked differences are apparent. Notably, the stacked matrilineal clusters of orangutan females contrast with the more spatially distributed maternal lineages in, for example, Coquerel’s dwarf lemurs, characterized by larger mean distances between the centers of activity of females of different clusters compared to those of the same cluster (Kappeler *et al.* 2002) Furthermore, within orangutan clusters, there is evidence that females are mainly first and second-degree relatives, comprising families of adult mothers and their adult daughters and sometimes the younger offspring of these, while the precise genealogical relationships of females in other non-gregarious species are often not known or taken into account.

Male-biased dispersal: evidence and comparisons

Males, in contrast to females, had a higher diversity of mtDNA haplotypes, most of which were sex-specific, and some of which were mutationally very distant. Interestingly, the number of new adult distinct females at a site reaches a stable plateau across years, but the number of new adult males identified continues to increase (van Noordwijk 2011a). Hence, further sampling in the future is expected to result in even more male specific haplotypes.

In addition, the shallow pedigree reconstruction showed that first-degree relatives among the males were rare in the study area. These results match the predictions for a model of male-biased dispersal. It was especially revealing that males did not have any mothers or maternal sisters in the study area, except in the case of one young individual. Since none of the males shared maternal ancestry with the well-known centrally located females from clusters B and C, our results indicate that the study site is not a natal area for any of the males. Furthermore, there were a number of rare haplotypes found in single males, highlighting their different maternal ancestry compared to the females. These male-specific haplotypes mirror the results of studies in the gray mouse lemur and Coquerel's dwarf lemur (Kappeler *et al.* 2002; Wimmer *et al.* 2002; Fredsted *et al.* 2004). Also, the large mutational differences for some male-specific haplotypes further suggest that the natal area of these particular males is geographically isolated or distant, although pinpointing the precise location requires further investigation.

Some males, however, belonged to haplotype A, characteristic of the peripherally ranging females. Thus it is possible that, unless haplotype A is extremely widespread, these males have their maternal relatives not too far from the study area, suggesting that they have travelled short distances. Since male home ranges are large and surpass the size of the study site, it is not fully clear whether the males with haplotype A have home ranges that include the natal area, and if so, whether this feature is permanent or temporary i.e. restricted to early stages of dispersal. Therefore for the males with the common haplotype A, there is a possibility that they have expanded their natal ranges but as of yet we have no confirmation of such an event.

In some cases, males shared their mtDNA haplotype with each other and could be maternally related, but parallel male dispersal is unlikely given low male sociality (Delgado & Van Schaik 2000). It is nonetheless possible for related males from the same maternal lineages to converge at a site if the dispersal options are limited due to forest fragmentation and other ecological barriers. This is unlikely to hold for Tuanan, but may be an important consideration elsewhere.

In sum, we find that while females are extremely faithful to their natal ranges, males tend to disperse. Our results also suggest possible variation in the distances travelled by males, with some travelling over longer distances and others perhaps originating from adjacent areas or clusters. The possibility that some males, perhaps the younger ones, move shorter distances from their natal areas or still encompass them needs further investigation.

The genetic findings of female philopatry and male-biased dispersal are in line with previous observational indications at several orangutan research sites (Mitani 1989; Galdikas 1995; van Schaik & van Hooff 1996; Delgado & Van Schaik 2000). Genetic evidence for this dispersal pattern has also been found at the Bornean site of Sabangau (Morrogh-Bernard *et al.* 2010), but this is the first orangutan study to examine the precise genetic and spatial relationships among a large set of individuals.

Factors affecting the power to disentangle dispersal patterns

Our investigation highlights the importance of several factors pertinent especially to non-gregarious species with slow life histories that affects the power to genetically discern female philopatry: sampling regime, life history traits, and spatial distribution. First, we were better able to resolve the pedigree of females whose home ranges were fully

encompassed within the study site (cluster B and C), compared to the relationships of females who partially ranged within the study area (cluster A). These findings are a strong indication that in non-gregarious species the sampling regime is of critical importance. While group-living species have cohesive distinct units of regularly interacting individuals that determine which individuals are sampled, the absence of such units in non-gregarious species means that sampling criteria are not often defined but rather of an opportunistic nature. Thus, as exemplified by the orangutans, the social network of non-gregarious individuals might not be accessed unless the entire home range of a female is fully incorporated in the sampling regime, leading to potentially erroneous inferences. Opportunistic genetic sampling without spatial information might partly account for discrepancies between behavioral and genetic results, especially where small sample sizes can lead to biases.

Second, life history traits have an influence on the number of close relatives in a species. The slow reproductive rate of orangutans should result in very few sets of first-degree relatives. Thus, the chances of sampling parent-offspring dyads and being able to infer sibships in this manner will strongly depend on the ages and survivorship of individuals. For instance, the relationship between two sisters might not be detected if the mother is not alive. These effects illustrate the importance of considering life history traits such as mean life expectancy, interbirth intervals, and other reproductive characteristics of a species, when examining dispersal patterns in species.

Third, we found stacked matrilineal clusters of females, whose home ranges overlapped. The substructure among females makes it difficult to assess relatedness, since there are both closely related dyads as well as unrelated dyads sharing the same area. This substructured spatial distribution might make estimates of average relatedness poor measures for female philopatry, and may be a confounding factor in previous genetic studies of orangutans. It could also explain cases in other species where despite behavioral and genetic evidence for female site fidelity, average relatedness for females are not higher than expected by chance, as in a study of cougars (Biek et al., 2005).

The different dispersal patterns found across orangutan populations could also be a result of intraspecific variation. Such variation would be indicative of facultative dispersal and a high degree of flexibility dependant on population density and habitat characteristics among others, affecting the balance between benefits and costs. In the dusky-footed woodrat, for example, evidence for female kin structures was strongest at intermediate population densities, leading the author to propose that "high densities erode kin structures in response to local competition" (McEachern et al., 2007). In the gray mouse lemur, despite female philopatry, there is also evidence for the occasional dispersal of females. This was suggested by the spatial conglomeration of females with diverse haplotypes and no obvious female structuring, as well as the presence of multiple clusters of females that were not in spatial proximity but shared the same haplotype (Fredsted *et al.* 2004). In gorillas, males can either remain in the natal group or leave, and the fitness consequences of dispersal decisions for males at least, have been shown to depend partly on demographic variables (Robbins & Robbins 2005). In chimpanzees, despite the general pattern of extreme male philopatry and female-biased dispersal, recent research shows great variation across sites as well as in time (Mitani *et al.* 2002; Nishida *et al.* 2003; Lukas *et al.* 2005). Whereas at sites such as Mahale and Taï almost all young females emigrate, at the site of Gombe only 50% do and at Bossou none at all. The difference at the latter two sites has been attributed to their lower population sizes and

greater isolation from other sites (Mitani *et al.* 2002; Nishida *et al.* 2003). Habitat characteristics appear to play a role in orangutans too. For instance, both Tuanan and Sabangau are located in rich peat swamp forests, where females may be able to accrue ecological and social benefits without having to modify their maternal range too much, while males can avoid inbreeding by dispersing. However, habitat fragmentation, as proposed for the study population in the Lower Kinabatangan Wildlife Sanctuary (Goossens *et al.* 2006b), could impede the normal movement of males. Another interesting possibility related to spatial range expansions is that of suitable unsettled habitat becoming available, especially when coupled with an unfavorable population density. Such an event could alter the benefits and costs of dispersal for females and males, for instance, by increasing the fitness of dispersers.

Having found strong evidence for the spatial clustering of genetically related female orangutans, the benefits of having relatives as neighbors requires further study. This is of special interest given the findings of Singleton & van Schaik (2002) on the preferential association among females that appeared to be related. While the genetic relationships of the females at this site were not confirmed, our findings for the Bornean population of Tuanan do show the presence of spatio-genetic clusters of related females with highly overlapping ranges, which may provide the opportunity for nepotistic associations within the female kin structures. Behavioral observations at Tuanan show that, despite the lower levels of sociality displayed by Bornean orangutans compared to Sumatran orangutans, and despite the generally low number of interactions among females, these do indeed take place. In fact, a study now underway shows that such interactions are more likely to occur among the females of a matriline (Van Noordwijk 2011b). These instances include encounters that provide opportunities for play behavior among the offspring of closely related females. These findings lend further support to the role given by Singleton and van Schaik (2002) to nepotistic tolerance in determining the nature of social interactions and opportunities to acquire new skills, particularly through play behavior. Nepotistic tolerance might also make settlement in overlapping home ranges easier for relatives than non-relatives. That the primary role of associations among females should provide social benefits such as learning was already suggested by van Schaik (1999). If these benefits are distributed mainly among relatives, kin selection could be an important evolutionary mechanism underpinning matrilineal kin structures in orangutans, warranting further investigation.

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4 Emerging patterns of sex-biased dispersal in orangutans: when field and genetic data do not agree

4.1 Abstract

The dispersal patterns of species affects population genetic structuring as well as the social interactions among individuals. Due to their lower sociality levels and absence of distinct recognizable social units, non-gregarious mammals have received less attention compared to group-living taxa, and thus remain underexplored. Among these non-gregarious species are orangutans, which present an enigmatic case due to their unresolved social organization, and discrepant behavioral and genetic results. The few genetic studies of current dispersal patterns show variation across sites that could indicate high intra-specific variation, or methodological differences. In order to clarify the dispersal patterns of orangutans, we examined five populations from Borneo and Sumatra, generating haplotypic data for the uniparentally inherited mitochondrial DNA (mtDNA) hypervariable region I (HVRI) and genotypic data for 17 biparentally inherited microsatellite loci. Using these data, we tested the predictions on mtDNA diversity, average pairwise relatedness estimates and genealogical relationships for various dispersal models. More varied maternal ancestries among males and the presence of close maternal relatives among females, as expected for a model of female philopatry and male-biased dispersal, were found. Contrary to our expectations, however, average relatedness estimates were not significantly higher for females compared to males. The discrepancy in the results produced by various genetic measures, as well as the contrast with field observations, result from biological and methodological issues, including female substructuring and sampling regime among others. Through the insights we obtained from the case of the orangutans, we discuss these issues, particularly relevant to non-gregarious species, highlighting their importance for future studies of relatedness and dispersal.

4.2 Introduction

Characterizing the dispersal patterns of species is a central theme in the study of social behavior and kin cooperation. But it also provides crucial insights to our understanding of population genetic structure (Avise *et al.* 1987; Clobert *et al.* 2001). In mammalian species, dispersal is often male-biased: males are more likely to leave or travel longer distances away from the natal area to breed, and females are more likely exhibit site fidelity when breeding, remaining philopatric (Greenwood 1980; Dobson 1982). The limited levels of gene flow associated with sex-biased dispersal (SBD) and high coancestry of philopatric individuals have an influence on population subdivision, especially when combined with polygynous mating. Consequently, SBD has effects on the maintenance and loss of genetic diversity, including the probability of fixation of beneficial alleles and hence, the potential of local adaptation (Avise *et al.* 1987; Storz 1999; Whitlock 2001).

While dispersal can be studied directly through field observations, this method provides data on the movement of individuals but not necessarily on effective dispersal, that is,

migration accompanied by successful reproduction, which leads to gene flow (Slatkin 1985; Prugnolle & de Meeus 2002). Furthermore, for species with long life histories, sufficiently long-term behavioral data are often unavailable, so the dispersal of some individuals might go undetected, particularly if delayed as a response to environmental changes. Also, simply examining individual movement does not allow disentangling the relatedness among individuals, necessary to explore the role of kin selection in social interactions. Thus, indirect methods such as genetic analyses have proven invaluable in investigating both long-term dispersal using measures of historical gene flow, and instantaneous dispersal, that is, within one generation, using measures such as relatedness estimation (Prugnolle & de Meeus 2002; Lawson Handley & Perrin 2007).

Historical sex-biased gene flow is ideally studied through the combination of non-recombining maternally inherited markers on the mitochondrial DNA (mtDNA) and non-recombining paternally inherited markers on the Y chromosome (Lawson Handley & Perrin 2007). Long-term female philopatry is expected to result in higher geographical structuring and differentiation at mtDNA than Y-chromosome markers (or than differentiation at biparentally inherited markers). By contrast, long-term male philopatry should lead to the reverse pattern. Alternatively, a snapshot of current dispersal is obtained through the comparative study of biparentally inherited markers in females and males (Prugnolle & de Meeus 2002). One widely used approach is the estimation of average pairwise relatedness at these markers, which is expected to be higher for the philopatric sex compared to the dispersing sex since related individuals remain in the natal area. Other statistical approaches also exist, for instance, the comparison of "assignment indices" and population structure measures through Wright's F-statistics, but these are most useful when the magnitude of sex-biased dispersal is extreme (Goudet *et al.* 2002).

These genetic measures have been widely used in mammalian studies, most particularly in their application to species that live in stable breeding groups and in which the role of kin selection underlying social behavior has prompted widespread attention. In a large proportion of group-living species, genetic studies have successfully confirmed behavioral observations on the direction of SBD (African lions; Avise *et al.* 1998; wild rabbits; Surridge *et al.* 1999). In other cases, the application of genetic tools has provided novel insights and challenged expectations from field studies alone. For example, in a study of black and white colobus monkeys (*Colobus guereza*) Harris et al. (2009) found closely related female pairs distributed in separate groups, although there was no observational evidence for female emigration. By combining field and genetic information, group dissolution prior to the start of behavioral observations emerged as the most parsimonious explanation. Furthermore, application of molecular techniques has permitted the detection of unusually low or high gene flow in particular populations that could be attributed to recent anthropogenic effects (Gibraltar macaques; Modolo *et al.* 2008; African elephants; Wittemyer *et al.* 2009).

Few studies have, however, examined non-gregarious species, which do not live in permanent social groups. In such species, it is difficult to identify a core set of individuals to examine, highlighting the obstacles of locational versus group-based study. The small number of genetic investigations of non-gregarious mammals focus on carnivores (raccoons; Ratnayeke *et al.* 2002; cougars; Biek *et al.* 2006; bears; Zedrosser *et al.* 2007), rodents (woodrats; Gould & Eldredge 1993; McEachern *et al.* 2007), and a few lemur species among the primates (Kappeler *et al.* 2002; Eberle & Kappeler 2006; Radespiel *et al.* 2009). These studies confirm the general mammalian pattern of male-biased dispersal

(MBD). Intriguingly, however, average relatedness estimates for the females do not always agree with expectations: in cougars (*Puma concolor*) for instance, female residents do not show higher values than expected by chance, while females of the banner-tailed kangaroo rat (*Dipodomys spectabilis)* differ across populations in the spatial scales at which they are found to be related, suggesting intra-specific variation (Gould & Eldredge 1993).

Among the most puzzling non-gregarious species are the orangutans, the highly endangered Asian great apes. Although orangutans like most other great apes show a fission-fusion social organization, they stand out as a result of their especially low levels of sociality (van Schaik 1999). Furthermore, there may be another striking contrast among these species. Behavioral studies point to extreme female philopatry and MBD in orangutans (Galdikas 1985a; Mitani 1989; van Schaik & van Hooff 1996; Delgado & Van Schaik 2000), whereas the African great apes as well as humans all show frequent female dispersal (Eriksson *et al.* 2006; Wilkins & Marlowe 2006; Douadi *et al.* 2007; Langergraber *et al.* 2007; Guschanski *et al.* 2008).

Nevertheless, the social organization and dispersal patterns in orangutans are unclear. Genetic studies of the mtDNA and Y-chromosome agree with historical MBD (Chapter 2; Arora *et al.* 2010; Nater *et al.* 2011b). Yet, three genetic studies focusing on current one-generation or instantaneous dispersal patterns in different populations produced conflicting results, indicating dispersal of both sexes (Utami *et al.* 2002), philopatry of both sexes (Goossens *et al.* 2006b), or MBD (Morrogh-Bernard *et al.* 2010). Intra-specific variation dependant on resource distribution and population density has been found in other species including chimpanzees (Mitani *et al.* 2002), so the discrepancies in orangutans could reflect extreme population differences. However, the variation could also stem from methodological differences among the studies.

Clarifying the dispersal pattern of orangutans is important for several reasons among others. First, sex-biased dispersal has profound implications on population genetic structure, determining how genetic variation is partitioned and the rate at which it is lost through drift (Sugg *et al.* 1996; Storz 1999). Second, it allows us to unravel the population history of orangutans, and disentangle past from present patterns of gene flow which help determine the impact of recent changes including human disturbances. These two reasons are linked to the conservation management of an endangered species. Third, a study underway shows that, despite the low level of associations among orangutans, these are more likely to take place among maternally related females than among unrelated individuals, providing opportunities for kin selection to operate (Eldredge *et al.* 2005; in prep.). Thus it is of special interest to determine whether female kin structures are widespread, or whether they differ considerably across populations, resulting in variation in the opportunities for nepotistic association in orangutans.

To elucidate instantaneous dispersal patterns in orangutans, we investigated genetic relatedness patterns across several long term study sites of orangutans from both the Bornean and Sumatran species (*P. pygmaeus* and *P. abelii*, respectively). We integrated genetic data a uniparentally inherited mitochondrial DNA (mtDNA) marker and 17 biparentally inherited microsatellite markers together with field data, so as to test predictions on the genetic outcome of four different dispersal models (Table 4.1). These models take into account the larger home ranges of orangutan males compared to females (Delgado *et al.* 2009), thus referring to male philopatry as male natal range expansion. The predictions were assessed through a combination of comparative analyses between

the sexes of mtDNA ancestry, average pairwise relatedness estimates and parentage-based pedigree relationships. We especially focused on maternal relationships because in orangutans, as in other mammals, the natal range is equivalent to the maternal range. To assess maternal ancestry, we used an mtDNA marker because previous findings showed variation within orangutan populations (Arora *et al.* 2010). Where one sex has a greater tendency to leave the natal area, immigrants are expected to originate from different maternal lineages and thus have high mtDNA diversity levels. In the case of MBD, mtDNA diversity levels should exceed those of the philopatric females, and unique male-specific haplotypes not shared with the philopatric individuals should be found. Furthermore, in sex-biased dispersal, the dispersing sex should have lower levels of average relatedness compared to the philopatric sex, and not comprise close same-sex relatives, unless there is parallel dispersal in which case mother-offspring pairs are in any case not expected. If females are philopatric, they should comprise close sets of maternally related dyads, but if males are philopatric they should comprise closely related maternal and paternal dyads. Despite the simple assumptions underlying our predictions, our results provide substantial evidence for a model of female philopatry and MBD across sites. In the light of our findings, we discuss the importance of biological and methodological considerations critical for studies of non-gregarious species.

Table 4.1 Predictions for four different models of dispersal in orangutans. Abbreviations: MBD (male-biased dispersal), FBD (female-biased dispersal), F (female), M (male), R (average pairwise relatedness estimates).

	Model	Description	Predictions*		
			mtDNA diversity	R	With close relatives
1	**Female philopatry and MBD**	F remain near maternal range, M move away; M from "foreign" maternal lineages expected	F<M	F>M	F>M (maternally related)
2	**Male (natal) range expansion and FBD**	M expand the maternal range and F leave	-	F<M	F<M
3	**F philopatry and M (natal) range expansion**	Both sexes remain at or near maternal range. M have larger home ranges than F. Thus, M are occasionally be sampled within natal ranges but not always	F~M	F~M	F~M relatives present
4	**F and M dispersal**	Both sexes leave the maternal range	F~M	F~M	F~M relatives rare‡

* F>M (higher for females than for males), F~M (similar for both sexes)

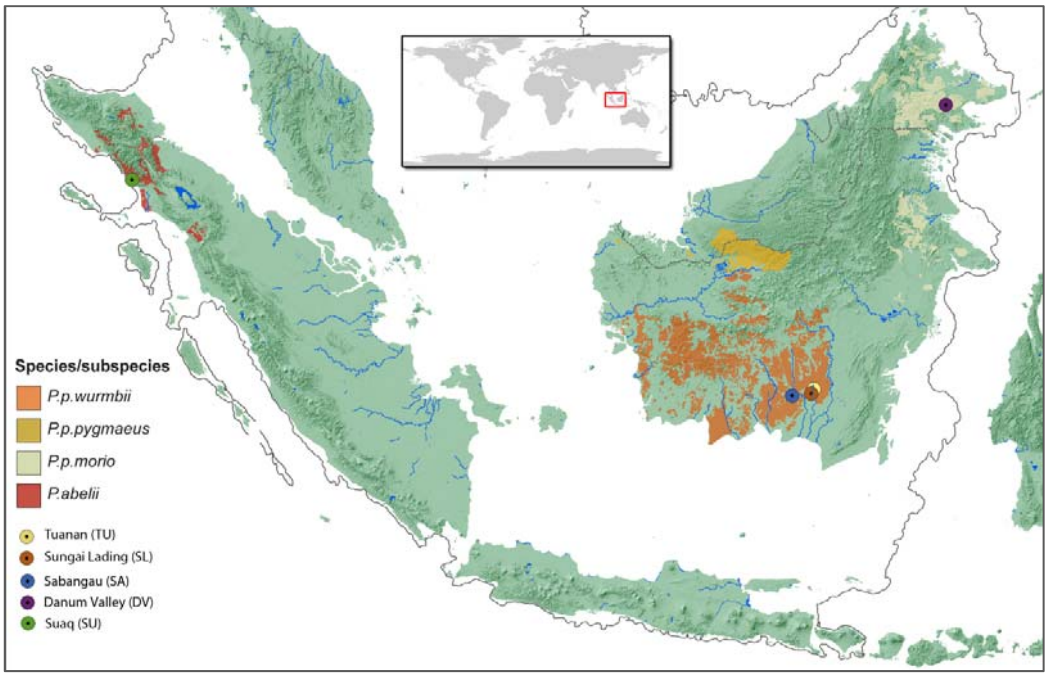
‡ In the case of parallel dispersal, adults might still comprise related dyads but in any case mother-offspring pairs will be rare

4.3 Materials and methods

Sample and data collection

We obtained non-invasively collected samples from Bornean and Sumatran orangutans from the following natural populations (Fig.4.1): Tuanan (TU), Sungai Lading (SL), Sabangau (SA), Danum Valley Conservation Area (DV), and Suaq Balimbing (Suaq).

Fig. 4.1 Sampling sites in Borneo and Sumatra. The colored circles represent long-term field sites from which non-invasively collected samples were obtained. Based on map by E.P.Willems.



We obtained genotypic and haplotypic data according to the protocols described in Arora et al. (2010). For all sites, data from 17 nuclear microsatellite markers were available (SI, Table 4.4). None of the markers showed evidence for allelic dropout and null alleles, deviation from Hardy Weinberg equilibrium (HWE) or linkage disequilibrium (LD) as demonstrated through tests conducted in ML-NullFreq (Kalinowski & Taper 2006a), Arlequin 3.11 (Excoffier *et al.* 2005) and Genepop (Rousset 2008b), respectively. To exclude re-sampled individuals, we performed identity analyses on Cervus 3.0 (Kalinowski et al., 2007; Marshall et al., 1998), and removed identical genotypes. This led to a total of 126 unique individuals from TU (n=41), SL (n=26), SA (n=17), DV (n=18) and SU (n =24). For all unique individuals we sequenced 450 bp of the mitochondrial DNA (mtDNA) hypervariable region I (HVRI) region.

MtDNA analyses

We assessed mtDNA diversity patterns, representative of maternal ancestry, using the HVRI. First, individuals sharing a haplotype were assigned to the same maternal lineage. Second, we compared mtDNA diversity levels in each sex by examining the nucleotide (π) and haplotype diversity levels (H_d) of females and males using DNAsp 5.1 (Librado & Rozas 2009). To illustrate the frequencies displayed by each sex and the relationships among the different haplotypes, a median joining tree was produced using Network 4.0 (Bandelt *et al.* 1999). Additionally, two males from two sites (TU and SA) were included in these analyses due to their distinct mtDNA haplotypes, even though no nuclear genotypes were obtained as a result of low DNA quality and quantity.

Microsatellite analyses between the sexes

Relatedness analyses

The behavioral observations of female philopatry at several sites predict higher relatedness among females than males. To avoid a bias resulting from the inclusion of individuals who are not old enough to disperse, we incorporated only sexually mature individuals estimated older than 10 years and ranging independently from their mothers ("post-dispersal" individuals). The resulting sample sizes were as follows: TU (n=32), SL (n=23), SA (n=16), DV (n=16), SU (n=18).

We initially assessed marker informativeness and estimator performance as detailed in the Supporting Information (SI, Materials and Methods). Next, we obtained average pairwise relatedness (r) coefficients for the females and males at each site. Two analyses were carried out. In the first of these, r was estimated for all same-sex adult individuals at a site. In the second of these, r was estimated for each set of same-sex individuals at a site sharing their mtDNA haplotype and thus maternal ancestry. The second analysis was performed because a previous fine-scale study (detailed in Chapter 3) has shown the presence of overlapping matrilineal clusters comprising maternally related females, so examining these separately across sites should be of use to discern female substructuring.

To calculate r coefficients we used the new triadic likelihood estimator (TrioML; Wang 2007). This estimator computes relatedness of a dyad in relation to a third reference individual in order to reduce errors stemming from identity-in-state rather than identity-by-descent. It further allows the specification of a genotyping error rate, and its estimates are bounded between [0-1], a more legitimate range than that of other estimators. Moreover, an evaluation using empirical and simulated data for seven different estimators showed that the TrioML produced overall the most accurate estimates (Wang 2007). For the analysis at each site, all adult individuals from that site only were used as the reference population for the background allele frequency calculation. This approach yields more accurate relatedness estimates that when including individuals from other differentiated populations to compute the background allele frequency (Wang 2011b). We compared the average relatedness between female dyads and male dyads and tested for significance through the bootstrapping option available in the software, involving random re-sampling of individuals from the observed dataset and comparison of the observed differences in the observed and re-sampled datasets. For illustration purposes, r estimates at each site were corrected for the population average, which was set to a value of 0.

In addition, for comparison purposes r values were also computed with three other estimators: i) the coefficient of Queller & Goodnight (1989), which is frequently used in the literature and ii) and the coefficients of Wang (2002) and Lynch & Li (Lynch 1988; Li *et al.* 1993) chosen on the basis of their performance in the estimator evaluation (see SI Materials and Methods).

Parentage-based pedigree analyses

The four models of dispersal make predictions on the presence of relatives for females and males. An evaluation of marker informativeness, which is recommended but often not conducted, revealed that pairwise estimates of relatedness using our set of markers were not powerful enough to correctly discern relationship categories such as half-sib and unrelated dyads, similar to the case in other studies (Diniz-Filho *et al.* 2008). Therefore, we conducted a "pedigree" reconstruction through parentage assignment and sibship inference, in order to distinguish the presence of first and second-degree relatives for the "post-dispersal" set of adult females and males.

First, we performed parentage analyses for all individuals including infants and adolescents using the likelihood based approach implemented in Cervus 3.0 (Kalinowski *et al.* 2007). We conducted 10,000 simulations in order to determine critical values of the log-likelihood score for a strict confidence level of 95% and a relaxed confidence level of 80%. The parameters for these simulations included estimates of genotyping error rates for each population that were conducted through the "repeat-genotyping" and "unintentionally re-sampled individuals" approaches described by Hoffman and Amos (2011a). Only sexually mature individuals old enough to have sired offspring were incorporated as candidate mothers or fathers. The proportion of candidate parents was estimated from field data, but given the large influence it has on the statistical significance of the results (Krützen *et al.* 2004), several values for this parameter (0.05, 0.08, and 0.10) were tested to check the robusticity of assignments. Parentage was assigned based on the 95% strict confidence level, and evaluated by checking whether parent-offspring pairs known from field data were correctly identified. Next, individuals sharing a mutual parent were assigned a sibship (minimally half-sibs). A maternal sibship was confirmed if the individuals shared their mtDNA haplotype. Finally, we examined the genealogical relationships among females and males at each site by comparing the number of individuals of each sex with a maternal or paternal relative. Relatives included parents and siblings. A Fisher's exact test whether females had higher maternal relatives compared to males, as expected in a model of female philopatry, was carried out.

4.4 Results

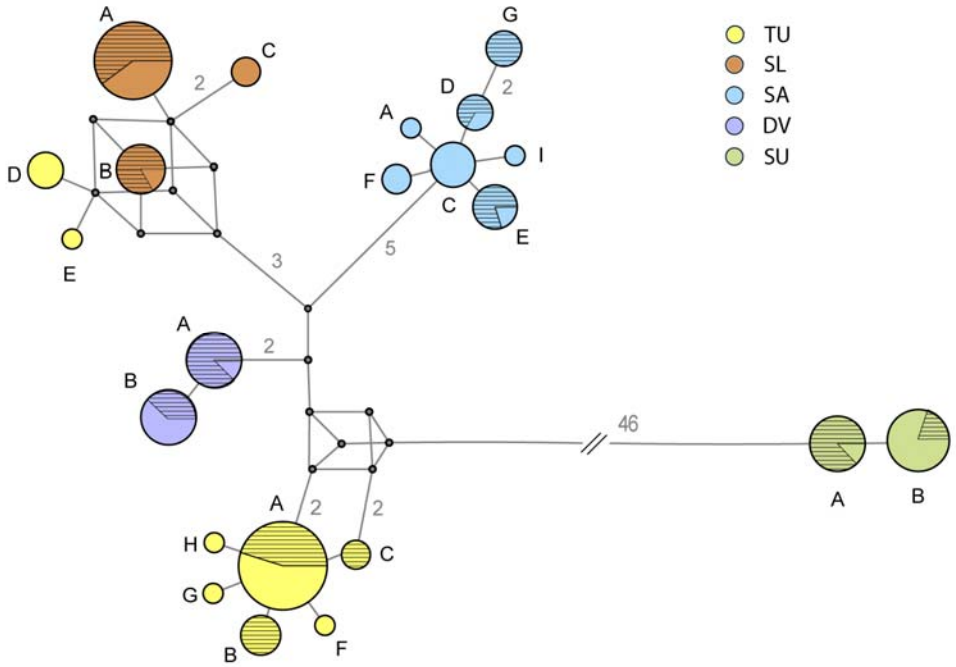
MtDNA analyses

In total, we found 22 different mtDNA haplotypes in Bornean and Sumatran populations, without any instances of sharing across sites. Three of the five sites showed higher haplotype diversity for males compared to females (Table 4.2). These sites were also characterized by sex-specific haplotypes (Fig. 4.2), particularly among males (TU: 5 out of 8 haplotypes; SL: 1 out of 3 haplotypes; SA: 4 out of 7 haplotypes). We also found haplotypes that were specific to females at TU (2 out of 8 haplotypes) and SA (1 out of 7 haplotypes).

Table 4.2 MtDNA diversity patterns of females and males of Bornean and Sumatran orangutan populations. The table shows, for each sex at each site, the number of haplotypes, haplotype diversity (H_d) and standard deviation (s.d.), and nucleotide diversity (π) as a percentage and standard deviation (s.d.). Abbreviations: N_F (number of females), N_M (number of males), F (females), M (males).

Pop	**No.**	**Haplotypes**		**$H_d\,(1-\sum f_i^2)$ ± (s.d.)**		**π(%) ±(s.d.)**	
	N_F, N_M	F	M	F	M	F	M
TU	15,18	3	6	0.590 (0.106)	0.621 (0.121)	0.149 (0.034)	0.986 (0.278)
SL	14,9	2	3	0.495 (0.088)	0.556 (0.165)	0.333 (0.059)	0.386 (0.121)
SA	6,11	3	6	0.733 (0.155)	0.800 (0.114)	0.419 (0.116)	0.236 (0.054)
DV	10,6	2	2	0.467 (0.132)	0.333 (0.215)	0.105 (0.030)	0.075 (0.048)
SU	9,9	2	2	0.389 (0.164)	0.222 (0.166)	0.087 (0.037)	0.050 (0.037)

Fig. 4.2 Sex-specific mtDNA haplotypes. Each circle represents a different haplotype, and each color corresponds to one of the four Bornean sites: Tuanan (TU), Sungai Lading (SL), Sabangau (SA), purple; Danum Valley (DV) and Suaq (SU). The proportion of males found with a haplotype is indicated through solid fills; the proportion of females through hatched fills. Haplotypes differ by one mutation unless specified otherwise.



Microsatellite data analyses between the sexes

Relatedness analyses

The TrioML estimator showed higher average pairwise relatedness (r) among females than males at most sites, although the differences between the sexes were, with the exception of Sabangau, not significant (Fig. 4.3). It must be noted, however, that there was a large variation in the results produced by the different estimators, except in the case of one study site (SI, Fig. 4.6Fig. 4.). In Sabangau (SA), female dyads consistently had higher r estimates than male dyads, irrespective of the estimator used, and despite the small sample size ($n_{female\ dyads} = 10$; $n_{male\ dyads} = 55$), indicating that the difference between the sexes at this site is robust.

We also estimated r for same-sex individuals sharing maternal ancestry (Fig. 4.4). In general, r estimates for females with the same mtDNA haplotype were higher than those obtained when all females at a site were pooled together. Thus, females from the same maternal lineage tended to have high biparental relatedness. Males sharing maternal ancestry, by contrast, did not necessarily show high biparental relatedness.

Fig. 4.3 Relatedness estimates for female and male dyads. Trio ML r values and standard errors corrected for population averages are shown for female dyads (black bars) and male dyads (gray bars) for Tuanan (TU), Sungai Lading (SL), Sabangau (SA), Danum Valley (DV), and Suaq (SU). Significant differences between FF and MM dyads are indicated by * ($p<0.05$) Estimates are based on 17 microsatellite markers for all sites.

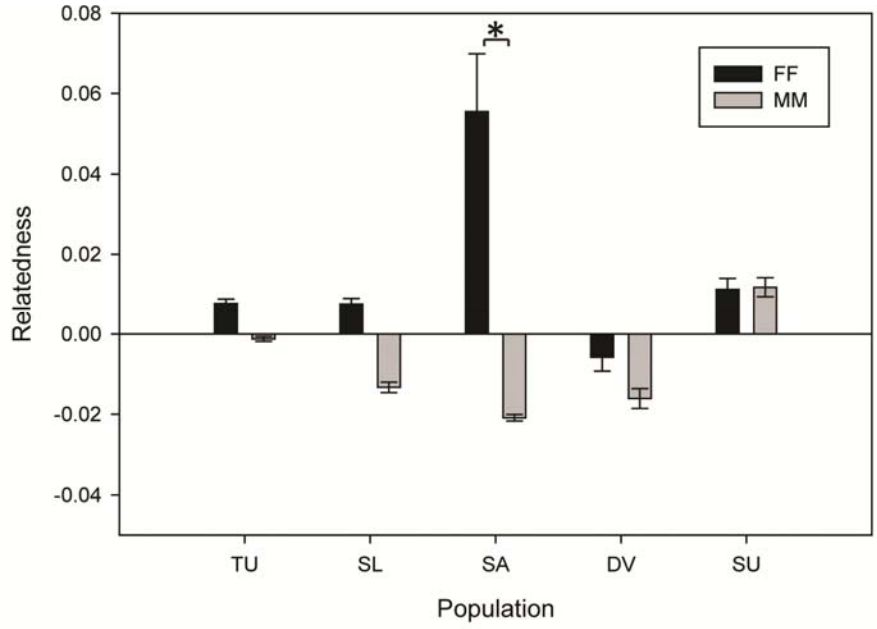
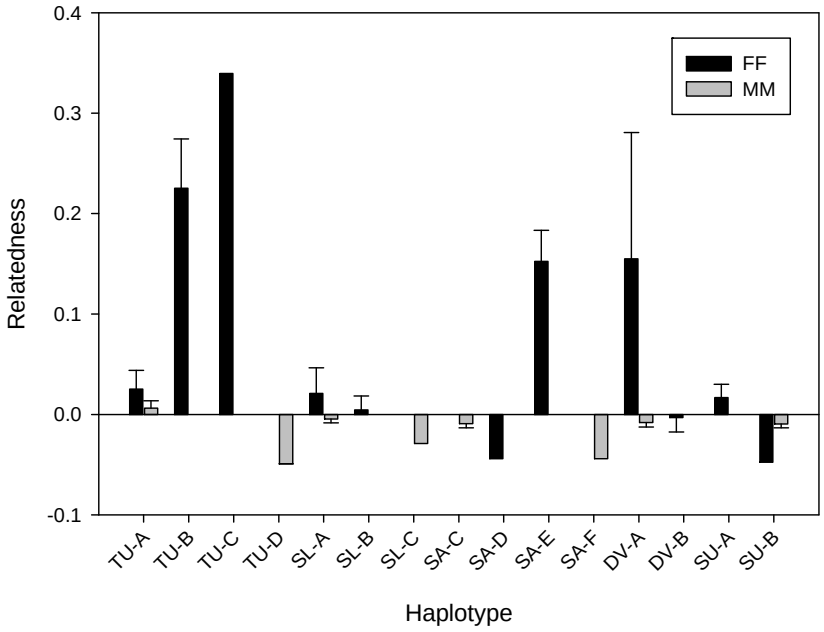


Fig. 4.4 Relatedness of females and males within maternal lineages. The Trio ML r estimates (corrected for population averages) and variances are shown for same-sex individuals sharing an mtDNA haplotype. Estimates were computed for female dyads (FF; black bars) and male dyads (MM; gray bars) at each site. Site-haplotype is indicated on the x-axis. Abbreviations for sites are as follows: Tuanan (TU), Sungai Lading (SL), Sabangau (SA), Danum Valley (DV), and Suaq (SU).



Parentage-based pedigree analyses

We used a parentage-based pedigree reconstruction to examine genealogical relationships and compare the patterns for females and males at each site in terms of the presence of relatives. With respect to maternal relatives, our results show that at most sites, more adult females than adult males had mothers and maternally related sisters present (Table 4.3). In Tuanan (TU), for instance, 10 out 15 females had at least one maternal relative within the study area (67%), whereas this was true for only 1 out of 17 males (6%). The differences between the sexes were either statistically significant or showed a trend in the direction predicted by a model of female philopatry and male-biased dispersal for 4 out of the 5 sites (Fisher's exact test, TU: $p < 0.001$, SL: $p>0.1$, SA: $p<0.05$, DV: $p = 0.06923$, SU $p = 0.01186$). As for paternal relatives, we confirmed none for the females or the males.

Table 4.3 Maternal and paternal relatives of females and males. For each sex, the number of individuals with at least one maternal relative, and the number of individuals with at least one paternal relative are shown (percentages from the total number of females or males at a site are given in parentheses). Abbreviations: N_F (total number of females at a site), N_M (total number of males at a site).

Site	N_F, N_M	With maternal relatives		With paternal relatives	
		Females	Males	Females	Males
TU	15, 17	10 (67%)	1 (6%)	0 (0%)	0 (0%)
SL	14,9	3 (21%)	2 (22%)	0 (0%)	0 (0%)
SA	6, 11	4 (67%)	1 (9%)	0 (0%)	2 (18%)
DV	9, 7	4 (44%)	0 (0%)	0 (0%)	0 (0%)
SU	9, 15	4 (44%)	0 (0%)	0 (0%)	2 (13%)

4.5 Discussion

We have examined current or instantaneous dispersal through comparisons of mtDNA diversity, average pairwise relatedness estimates (r) and genealogical relationships between the sexes. At several sites, we detected the presence of immigrant males originating from "foreign" maternal lineages, that is, maternal lineages different to those of the females at a given site. The analyses of parentage and inferred sibships showed that females often comprised closely related maternal dyads, whereas few males had maternal or paternal relatives. Although males at a site sometimes shared maternal coancestry, association among them is rare. Thus, our findings are in agreement with a model of female philopatry and male-biased dispersal, concurring with behavioral observations and genetic studies of historical gene flow (Arora *et al.* 2010; Nater *et al.* 2011b). However, contrary to our expectations, r was not significantly higher for females compared to males. In addition, we occasionally found maternal relatives for the males. Why do we find these mixed results? In the following we discuss the effects of biological and methodological factors on two genetic measures of instantaneous dispersal: the conventional estimation of r, and parentage-based pedigree reconstruction. Building on the case of orangutans, we offer guidelines for future studies of relatedness and dispersal of non-gregarious species.

Biological factors

Delayed or restricted dispersal

Mothers were assigned to males at three sites (TU, SL, and SA). At most sites males were young, and in all cases unflanged. At one of these sites, SL, one of the two males with an assigned mother was rarely observed, and so may have been in the early stages of dispersal. It is possible then that some of these males have not yet left the natal area. Another alternative is that these males have expanded their natal ranges, occasionally being found with their maternal relatives. Yet the rarity of maternal assignments for individuals sampled across years makes this scenario unlikely. Another important consideration is the presence of barriers to dispersal. The site of SL has been subject to heavy environmental disturbances through forest fires, which are expected to result in fragmented habitats with limited dispersal options, especially for individuals that would otherwise display higher mobility. An environmental barrier to dispersal was posited by

Goossens et al. (2006b) as a possible explanation for the similarly high r coefficients found among females and males at the fragmented site they examined in the Lower Kinabatangan Wildlife Sanctuary. While it remains possible that some males do indeed remain in the maternal range, the few males with assigned mothers probably either delayed dispersal, or suffered reduced mobility as a result of habitat fragmentation.

Dispersal of related individuals

Even if dispersal is male-biased it is still possible for males at a site to be related, paternally or maternally. As occurs in African elephants, orangutan males compete for access to the maximal number of females, and given the larger home range sizes of males compared to females, paternal siblings are expected to be more spatially scattered than maternal siblings (Archie *et al.* 2008; Delgado *et al.* 2009). While the low associations among males make coordinated parallel dispersal unlikely (Delgado & Van Schaik 2000), a scenario of high male reproductive skew would increase the chances of encountering paternal relatives at a given site. Maternal or paternal relatives might also migrate to the same location as a result of life-history traits, dietary preference or the limitations imposed by habitat fragmentation. In western gorillas, for instance, females generally transfer to neighboring groups where they may be found with other related females (Bradley *et al.* 2007). This behavior has been attributed to the high male reproductive skew within a group, which results in paternally related female siblings of the same age-cohort that transfer around the same time, consequently settling in the same groups (Bradley *et al.* 2007). In mountain gorillas, female preference for natal habitat and diet has been proposed to explain the short distances travelled by females compared to males (Guschanski *et al.* 2008). Such a preferential movement is expected to also result in incidental relatedness among immigrants. The implication deriving from the dispersal of related same-sex individuals is that there might not be a significant different in r estimates between the philopatric and the dispersing sex. Coupled with the methodological factors affecting average r estimates, dealt with below, r estimates might therefore not be sufficiently sensitive to disentangle sex-biased dispersal. This shortcoming could explain why some orangutan populations show higher r coefficients among females than males, but the difference is not significant.

Life history traits

Life history traits including mating system, reproductive skew and inter birth intervals have genetic consequences on the levels of relatedness among individuals. First, low promiscuity and high male reproductive skew should lead to higher levels of coancestry and relatedness in the philopatric sex, whereas high promiscuity and low reproductive skew should result in lower coancestry and relatedness. In chimpanzee communities for instance, the presence of several paternal lineages may have lowered r estimates among philopatric males (Lukas *et al.* 2005). In fact, low male reproductive skew should result in a rapid reduction of biparental relatedness between individuals across generations, particularly if a female has offspring with multiple males. For example, two female orangutans whose mothers are related at the half-sib level have a true relatedness between them of 0.0625, only slightly above than an unrelated pair. Hence, even small biases in the estimation of relatedness due to reduced sample sizes or low marker quality and quantity might be problematic. Second, inter-birth intervals determine reproductive output and therefore, the number of related individuals at a given point in time. In species with fast life histories and large litter sizes, individuals should have, barring high

mortality, a large number of relatives across and within the same generation. But orangutans have extremely slow life histories, the slowest among non-human primates. As a result of their long generation times and long inter birth intervals, we expect individuals to have few close relatives, which will be reflected in pedigree reconstructions and estimates of r.

An additional noteworthy point relates to the variation in life history traits between the Bornean and Sumatran species. Behavioral evidence on mating behavior suggests that Bornean orangutans might have moderate reproductive skew compared to Sumatran orangutans (Van Schaik & Dunkel 2011). This difference is expected to result in comparatively lower levels of coancestry and relatedness in Bornean orangutans, and hence limit the benefits of female philopatry through nepotistic cooperation. To test these predictions, however, further data on association between individuals, paternity concentration, and additional Sumatran populations are required.

Overlap of maternal lineages

Our results show that in orangutan populations, different maternal clusters or lineages comprising related females can be found at a site, overlapping in their home ranges. Pooling together the females from different clusters lowers r estimates because individuals between clusters are not maternally related, although this effect might be partly counterbalanced by paternal relatedness across clusters. The higher the population density, and the greater the overlap of home ranges, the greater the downward bias in estimates of r of the philopatric sex, since more individuals from varying lineages will be included. Therefore, ascertaining the possible substructuring, for instance through mtDNA or Y- haplotype sharing in female and male philopatric species, respectively, should be of great use.

Methodological factors

The key issue of spatial scale in non-gregarious species

A critical question in any study of relatedness is that of the sampling unit, i.e. the focal set of individuals that is analyzed. For gregarious species characterized by permanent social groups, studies investigate the difference in same-sex relatedness within and between social groups (long-tailed macaques; de Ruiter & Geffen 1998; ie. guerezas; Harris *et al.* 2009). In some non-gregarious species such as the grey mouse lemur (Eberle & Kappeler 2006), individuals may nonetheless aggregate at sleeping sites, and thus the focal groups for analyses of relatedness are the sleeping groups.

However, among many non-gregarious species, adults are generally observed alone or with dependent offspring, with little or no association with other adults. This social structure raises the question of which particular set of individuals to analyze. Here several points are critical. First, in many studies, the field site delineates the sampling area. This strategy may be applicable to territorial species, but not for nomadic species in which individuals move unpredictably. Similarly, within species, demarcating the sampling area permits the study of individuals with stable home ranges but information on wandering individuals will be limited. An interesting case is provided by a study in the solitary banner-tailed kangaroo rats. Despite field observations of both female and male philopatry, offspring were not inbred, suggesting that females were not mating with

neighboring males (Avise & Wollenberg 1997). These results indicate that there may be wandering individuals temporarily in search of breeding opportunities, which might be excluded from analyses unless they are sampled.

Second, the size of the sampling area relative to the size of the home ranges of the subjects is critical in the study of non-gregarious species. In those in which individuals have small home range sizes and small dispersal distances, relative to sampling area, including all individuals encountered might result in low r coefficients among females and males even when there is sex-biased dispersal. One solution in this case is to measure genetic relatedness against spatial distance (Prugnolle & de Meeus 2002), as has also been done for various group-living species (ie. red deer, Nussey *et al.* 2005). To date, studies of a number of non-gregarious small-distance travelling mammals show, in agreement with patterns of female philopatry, the expected decrease in female r estimates with increasing genetic distance, and little or no distance effect for males (Coquerel's dwarf lemurs; Kappeler *et al.* 2002; raccoons; Ratnayeke *et al.* 2002; Quail ridge woodrats; McEachern *et al.* 2007).

Conversely, some non-gregarious species have extremely large home range sizes, and a field site might encompass few complete ranges, but still enable sampling of many individuals some of which range only partially within. This key aspect is illustrated by our fine-scale study at Tuanan, as detailed in Chapter 3. Orangutan females at Tuanan have home ranges estimated around 250-300 ha, while the total study area is composed of ca. 750 ha of grid-based trails. Our results here showed that for those females whose home ranges were mostly encompassed by the study area, we were able to disentangle their genealogical relationships, providing evidence for highly related dyads. By contrast, for the females who ranged only partly within the study area, we could determine the relationships among just a few of them, most probably because their relatives ranging outside the study area were not sampled.

For some species, researches could also expand their sampling area to encompass individuals who are not encountered at field sites. This is a difficult strategy for orangutans given the logistical difficulties imposed on sampling by an arboreal environment. But for several bear species, which can travel distances over 100 km/month (brown bears; Støen *et al.* 2005; black bears; Costello *et al.* 2008; polar bears; Zeyl *et al.* 2009), this approach has been possible, in conjunction with trapping and information of capture positions or satellite telemetry. Nonetheless, even widely distributed capture locations might not encompass the entire scale at which dispersal occurs (Zeyl *et al.* 2009). Furthermore, some capture locations may simply reflect unusual mobility of individuals away from their home ranges for specific purposes, such as the higher mobility of orangutan females when in search of mating opportunities (van Woerden & Pettersson 2007). For this reason, while capture locations are a good alternative, they are not as informative as home range data, especially when sample sizes are small and capture locations do not represent patterns of residence. These observations illustrate that the opportunistic locational sampling often applied to non-gregarious species may be problematic, and needs to be combined with behavioral and spatial information.

In the case of the orangutans, there are considerable differences across sites not only is study area size but also the total time span during which sampling has been conducted, resulting in variation in sampling intensity. Such sampling intensity is important, as illustrated by recent analyses at Tuanan that show that the number of new males

identified, and hence the potential number of new haplotypes, continues to increase over the years, while the number of new females identified reaches a maximum after a few years (van Noordwijk 2011a). Hence, at DV, where few individuals have been genetically characterized, further sampling over larger spatial and temporal scales could still produce a different picture.

Estimation of average pairwise relatedness

Average pairwise relatedness estimates (r) are often used to assess the degree of relatedness within a sex, as well as to discern relationship categories of dyads such as parent-offspring (PO), full-sib (FS), half-sib (HS), and unrelated (U). There are a number of estimators available to calculate r, and their performance is strongly influenced by marker quantity and quality, including number of unlinked markers, levels of polymorphism, and the frequency distribution shape of alleles (Van de Casteele *et al.* 2001; Blouin 2003; Csillery *et al.* 2006).

We assessed the discrimination power of the markers available in orangutans in distinguishing relationship categories, as recommended by Blouin (2003), In particular we focused on half-sibs, since we expect some siblings to share only one parent, and unrelated dyads. Unfortunately, our simulation results show that irrespective of the estimator used, there is a wide overlap between the latter two relationship categories, making their discrimination through r estimates prone to large errors. Csilléry et al. (2006) have also highlighted the same problem for the low relationship categories analyzed in five natural populations of vertebrate species, and also argued from their data that only parent-offspring and FS dyads might be discerned from the other categories using r estimates.

As for comparisons of r between the sexes, for most populations we found considerable variation in the results provided by different estimators, cautioning against their usage to make conclusive statements. Importantly, interpretations regarding philopatry and dispersal made on the basis of the absolute r estimates are not reliable, despite their occurrence in the literature. This is because absolute values are very sensitive to reference allele frequencies. When using other differentiated populations to compute reference allele frequencies, r estimates in the focal population will be high, reflecting the incorporation of unrelated individuals from more distantly related populations. Consequently, these absolute values might lead to erroneous interpretations of philopatry of both sexes. Therefore, it is necessary to consider the average for the focal population, and make inferences based on the comparative analyses between the two sexes.

Considerations in the usage of genetic measures of instantaneous dispersal

We have discussed several issues affecting the results we obtain from genetic measures of instantaneous dispersal in non-gregarious species. Particularly the conventionally used estimates of r show great sensitivity to a number of aspects. First, even if there is a sex-bias in dispersal, and immigrants leave their natal areas, they might show high relatedness. Some causal factors include parallel dispersal, high reproductive skew or limited movement as a result of habitat fragmentation, among others. So a prediction of higher relatedness among philopatric individuals compared to dispersing individuals is clearly too simplistic. We need to look at the relationships between females and males. It is advisable to combine r estimation with other measures. For example, investigation of

maternal and paternal ancestry, whenever the diversity of mtDNA and Y-markers allows, and a pedigree reconstruction should help clarify the relatedness among individuals.

Second, the slow life histories of some species such as orangutans and other great apes lead to small sets of closely related individuals at a given time. Levels of relatedness will also vary depending on reproductive skew, with higher coancestry among the offspring sired by a male with high mating monopolization for instance. It is to be expected then that r estimates decrease with the number of individuals included in an analysis, as observed in another study (Lukas *et al.* 2005).

Third, as we have seen in our studies of orangutans, several matrilineal clusters may overlap in their ranges, also resulting in underestimates of r among females. Consequently, the need to disentangle these subsets becomes clear.

Fourth, the size of the sampling area in relation to home range sizes is critical, and can strongly influence our determination of relatedness patterns. Including individuals whose home ranges are partly within a study area but are not fully encompassed will also reduce the power to detect philopatry if these individuals have their relatives elsewhere.

Fifth, and finally, our in-depth investigation of marker informativeness and estimator performance highlights the high sensitivity of r estimates to marker quantity and quality, as well as the set of individuals used in determining reference allele frequencies, and the importance of considering these points carefully. The small sample sizes of orangutans and other non-gregarious species could also lead to stochastic biases in estimates.

Our study of dispersal and relatedness patterns across orangutan populations capitalized on the integration of behavioral and spatial data as well as various genetic measures of instantaneous dispersal. The results from this integrated approach indicate a model of female philopatry and male-biased dispersal in orangutans, demonstrating that both historical and contemporary gene flow necessitates male mediation. Furthermore, our findings suggest that the discrepant patterns found to date are most probably a result of several biological and methodological issues and not to intra- or inter- species variation. These discrepancies were observed in the estimates of r, which are highly sensitive to life history traits, sampling strategy, and issues concerning the quantity and quality of markers as well as estimator performance. Thus, these estimates are not very reliable, and might also explain the disjunction of behavioral and genetic results in other species. Our findings illustrate the need, particularly in non-gregarious species with slow life histories, large home ranges, and small sample sizes, to produce several lines of evidence to disentangle instantaneous dispersal, as done for this study.

4.6 Acknowledgments

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4.7 Authors and affiliations

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4.8 Supporting Information

Materials and Methods

Marker informativeness and estimator performance

Recent analyses have shown that different relatedness estimators vary in their precision depending on number of markers, levels of polymorphism, allele frequency distributions, and population composition (Van de Casteele *et al.* 2001; Csillery *et al.* 2006; Wang 2006). Furthermore, the available estimators also perform differently depending on the true relatedness being assessed (Csillery *et al.* 2006; Wang 2006).

Prior to estimating relatedness of females and males, we assessed the information content of the markers and simulated their overall power for relatedness estimation using KinInfor v1.0 (Wang 2006). First, we examined two measures of information content: i) I_r, the informativeness of relatedness as a continuous measure of identity by descent, and ii) I_R, the informativeness of discrete relationship categories. Our specifications were parent-offspring (PO) and unrelated (U) dyads as the primary and null hypothetical relationships, respectively, a Dirichlet distribution of (1, 1, 1) that takes into account uncertainty in the distribution of relationships, and a significance level of 0.05. Next, we conducted iterative simulations of 100,000 dyads on KinInfor to quantify the power of the highest ranking marker, the second-highest ranking markers, and so on, until we had assessed the entire set of 17 markers. For these simulations we evaluated the discrimination power of half-sib (HS) versus U dyads, and PO versus U dyads.

In order to choose a suitable estimator we used KinInfor to compute the multilocus reciprocal of the mean squared deviations (RMSD) of relatedness estimates, which measures marker information according to estimator and allows choice of the most suitable estimator. Additionally, we carried out simulations in Coancestry v.1.0 (Wang 2011a) to assess the performance of seven different relatedness estimators on four relationship categories: parent-offspring dyads (PO), full-sib dyads (FS), half-sib dyads (HS) and unrelated (U) dyads. The following estimators were evaluated: the new triadic likelihood estimator (Wang 2007), a dyadic likelihood estimator (Milligan 2003), and the

moment estimators of Wang (2002), Lynch & Li (Lynch 1988; Li *et al.* 1993), Lynch & Ritland (1999), Ritland (Ritland 1996), and Queller & Goodnight (1989).

Results

Marker informativeness and estimator performance

We assessed the informativeness of each marker in discriminating relatedness categories and estimating relatedness. Given the high similarity in results across sites, we only present those for the site with the largest sample size (TU). Based on the I_R ranking (SI, Table 4.5), we further conducted iterative simulations of dyads of different relationship categories to analyze the power discrimination of different marker sets. Our results show that the set of 7-8 markers with highest I_R are very powerful in discriminating parent-offspring (PO) dyads from unrelated pairs (U). By contrast, even the usage of all 17 markers available only provides 54% power in distinguishing half-sib (HS) from U pairs (SI, Fig. 4.5). This lack of power is in all likelihood due to the skewed allele frequency distributions of these markers. Indeed, Wang (2006) has shown that 13-14 markers with 10 alleles uniformly distributed in frequency are sufficient to correctly distinguish 80% of HS dyads from unrelated dyads.

In terms of estimator performance, our results indicate that the multilocus reciprocal of the mean squared deviations (RMSD) is highest for the Lynch & Li (LL) and Wang (W) estimators, pointing to their higher precision, compared to the Lynch & Ritland (LR), Queller & Goodnight (QG) and Ritland (R) estimators, in estimating relatedness (SI, Table 4.6). The RMSD per marker for each estimator is provided in Table 4.7 (SI). Simulations in Coancestry for each site confirm the analytical results of KinInfor. While the different estimators can reliably discriminate PO or FS pairs from unrelated pairs, this is not the case for HS and unrelated pairs. The means, variances, and frequency distributions for the different relationship categories simulated are shown in SI, Table 4.8 (due to the similarity in results, data are shown for TU only).

Table 4.4 Nuclear microsatellite markers and primer pairs amplified in the study.

Marker	**Sequence (5'-3')**	**Repeat**	**Reference**
D1S550	F: CCTGTTGCCACCTACAAAAG	Tetranucleotide	(1)
D1S550	R: TAAGTTAGTTCAAATTCATCAGTGC	Tetranucleotide	(1)
D2S1326	F: AGACAGTCAAGAATAACTGCCC	Tetranucleotide	(1)
D2S1326	R: CTGTGGCTCAAAAGCTGAAT	Tetranucleotide	(1)
D3S2459	F: CTGGTTTGGGTCTGTTATGG	Tetranucleotide	(1)
D3S2459	R: AGGGACTTAGAAAGATAGCAGG	Tetranucleotide	(1)
D4S1627	F: AGCATTAGCATTTGTCCTGG	Tetranucleotide	(1)
D4S1627	R: GACTAACCTGACTCCCCCTC	Tetranucleotide	(1)
D13S765	F: TGTAACTTACTTCAAATGGCTCA	Tetranucleotide	(1)
D13S765	R: TTGAAACTTACAGACAGCTTGC	Tetranucleotide	(1)
D16S420	F: ATTTCCTGAGGTCTAAAGCACCC	Dinucleotide	(1)
D16S420	R: TTAGGCCCAGTCCACACTCAAG	Dinucleotide	(1)
D6S501	F: GCTGGAAACTGATAAGGGCT	Tetranucleotide	(1)
D6S501	R: GCCACCCTGGCTAAGTTACT	Tetranucleotide	(1)
D5S1457	F: TAGGTTCTGGGCATGTCTGT	Tetranucleotide	(1)
D5S1457	R: TGCTTGGCACACTTCAGG	Tetranucleotide	(1)
O4_6	F: GGCAATGTAACATATCCCTCTGTGT	Tetranucleotide	(2)
O4_6	R: AGCCATGGACCTTGTGAGAAAAG	Tetranucleotide	(2)
O4_A5	F: ATGGGCCCAGAAAACAACTCAGT	Tetranucleotide	(2)
O4_A5	R:AGATAAAGGAATGGATAGATGGACAGA	Tetranucleotide	(2)
O4_B5	F: GAGCCCTGATTCGTTTTACTGG	Tetranucleotide	(2)
O4_B5	R: AGCAAAGGCAGAAAACTGTAATGA	Tetranucleotide	(2)
O4_A7	F: ACTGGCCCATTCAAAGTCTGTCATT	Tetranucleotide	(2)
O4_A7	R: ACTGGCCCATTCAAAGTCTGT	Tetranucleotide	(2)
O4_A1	F: CTCCCCTTCCTTCCTTTATTCAGTT	Tetranucleotide	(2)
O4_A1	R: CAACACTTGGCAGTCACAAATCAG	Tetranucleotide	(2)
O4_B17	F: GTACCGACGGTGCACGAACAATGTA	Tetranucleotide	(2)
O4_B17	R: AGCCTGGCTGAAAAGTGGAACTGAG	Tetranucleotide	(2)
O4_B20	F: CCTGCATTTTGTCACTCCCTCAACC	Tetranucleotide	(2)
O4_B20	R: CTGCCACACCTCCATGGACACAGAT	Tetranucleotide	(2)
O4_C13	F: CTGGGCACACTGTATATGGGGTAG	Tetranucleotide	(2)
O4_C13	R: GTTTGAGACCACTCATGATGCAAAGACC	Tetranucleotide	(2)
O4_C9	F: TGCAGGCCAGGGCTTCTTTCAA	Tetranucleotide	(2)
O4_C9	R: CAGTCTCCCCAGGACCCCTACACAG	Tetranucleotide	(2)

[1] References:

(1) Goossens B, Chikhi L, Jalil MF*, et al.* (2005) Patterns of genetic diversity and migration in increasingly fragmented and declining orang-utan *Pongo pygmaeus* populations from Sabah, Malaysia. *Mol Ecol* **14**, 441-456.

(2)Nietlisbach P, Nater A, Greminger M, Arora N, Kruetzen M (2010) A multiplex-system to target 16 male-specific and 15 autosomal genetic markers for orang-utans (genus: *Pongo*). *Conservation Genetics Resources*.

Table 4.5 Measures of informativeness, allelic diversity and power for each marker: the table shows the ranking of I_R (informativeness for inferring relationship categories), ranking of I_r (informativeness for estimating relatedness), N_A (number of different alleles), N_E (number of effective alleles), I (Shannon's Information Index), H_E (expected heterozygosity), P_A (analytical power), and P_s (simulated power) computed using Kininfor and Genalex. Data shown is for one site, TU, given the high similarity in results across sites.

	Informativeness		Allelic diversity				Power	
Marker	I_R	I_r	N_A	N_E	I	H_E	P_A	P_S
D3S2459	1	1	6	5.158	1.704	0.806	6	6
O4B17	2	2	6	4.489	1.619	0.777	5	5
D5S1457	3	3	5	4.255	1.501	0.765	7	7
D1S550	4	4	6	3.378	1.433	0.704	1	1
D6S501	5	5	7	3.117	1.413	0.679	2	2
O4A5	6	7	6	2.932	1.336	0.659	3	3
O4A1	7	6	4	3.636	1.335	0.725	12	12
O4B5	8	8	5	2.455	1.150	0.593	4	4
O4C9	9	10	4	2.691	1.147	0.628	9	9
D13S765	10	9	4	2.753	1.143	0.637	8	8
D4S1627	11	12	4	2.359	1.049	0.576	10	10
O46	12	11	3	2.594	1.018	0.614	14	14
O4C13	13	13	4	2.299	0.940	0.565	11	11
D2S1326	14	14	3	2.149	0.910	0.535	15	15
D16S420	15	15	3	2.071	0.883	0.517	13	13
O4B20	16	16	2	1.461	0.495	0.316	17	17
O4A7	17	17	2	1.260	0.360	0.206	16	16

Fig. 4.5 Discrimination power of markers available. Simulations of half-sib (HS) versus unrelated dyads, and parent-offspring (PO) versus unrelated dyads show the increase in discrimination power with increase in number of markers used, added according to their rank in informativeness (data shown for TU, due to the similarity in results across sites).

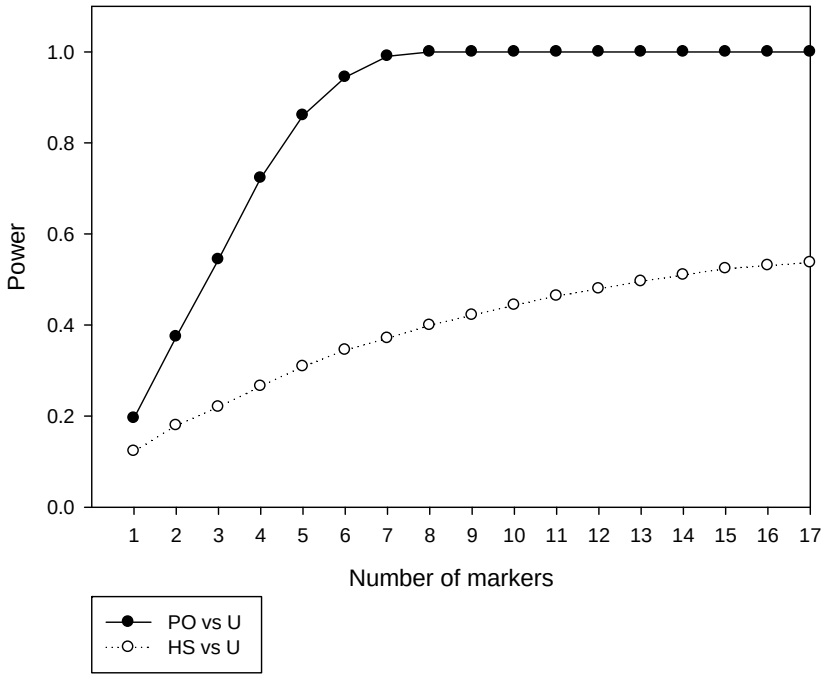


Table 4.6 Multilocus RMSD for five different relatedness estimators: Lynch & Li (LL), Queller & Goodnight (QG), Ritland (R), Lynch and Ritland (LR), and Wang (W) informativeness (data shown for TU, due to the similarity in results across sites).

	W	LL	LR	QG	R
Multilocus RMSD	42.3515	42.0778	41.2575	36.5817	21.6913

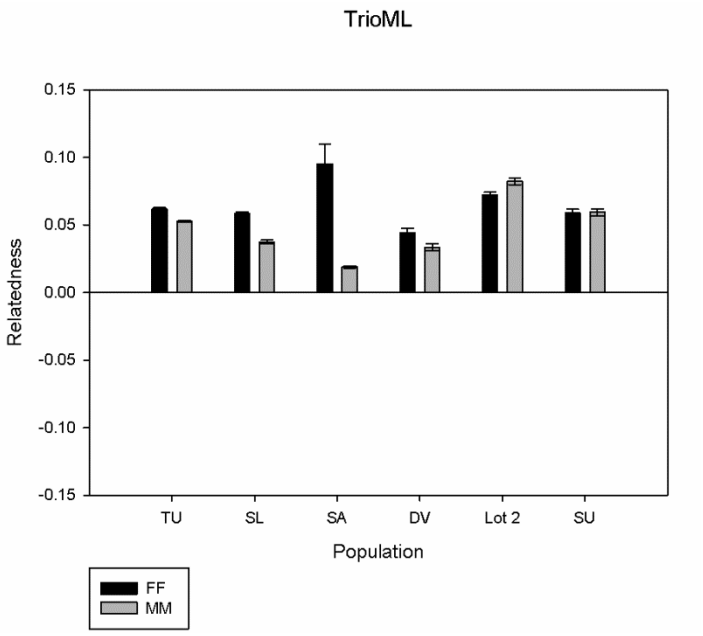
Table 4.7 Ranking of RMSD and the parentage exclusion probability for each marker. Estimators evaluated: Lynch Li (LL), Queller & Goodnight (QG), Ritland (R), Lynch & Ritland (LR) and Wang (W).

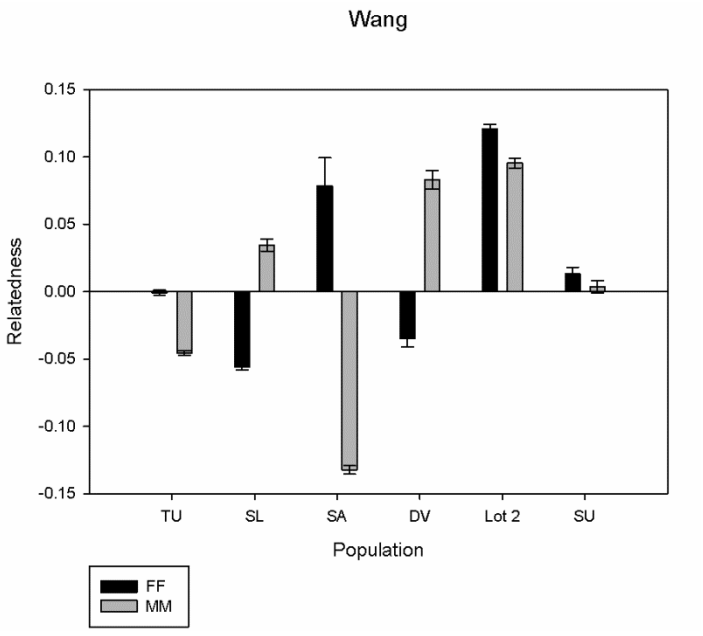
	RMSD					
Marker	**LL**	**QG**	**R**	**LR**	**W**	ExclP
D3S2459	1	1	1	1	1	1
O4B17	2	2	3	2	2	2
D5S1457	3	5	4	3	3	3
D1S550	5	0	5	7	5	5
D6S501	6	5	13	4	6	6
O4A5	7	7	12	6	7	7
O4A1	4	4	2	5	4	4
O4B5	11	9	11	8	10	10
O4C9	8	8	7	9	8	8
D13S765	9	10	8	11	9	9
D4S1627	12	11	14	10	12	12
O46	10	12	6	13	11	11
O4C13	14	15	16	16	14	14
D2S1326	13	13	9	12	13	13
D16S420	15	14	10	14	15	15
O4B20	16	16	15	15	16	16
Tetra	17	17	17	17	17	17

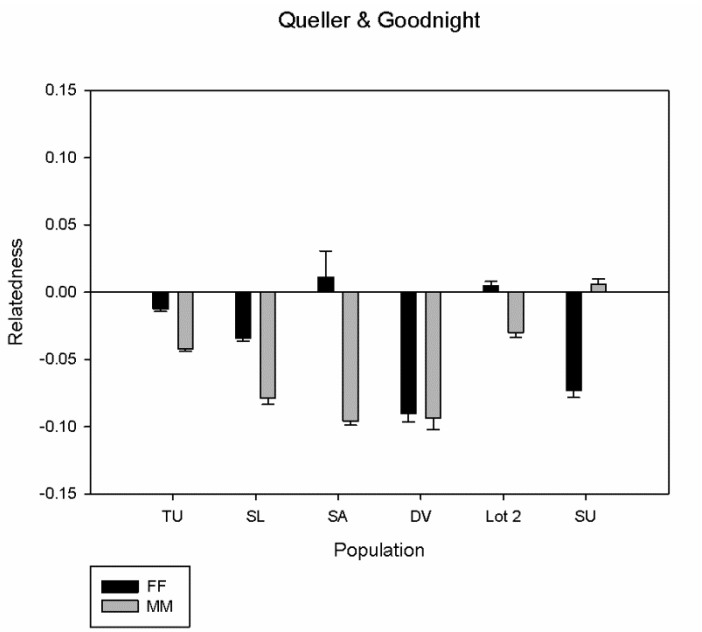
Table 4.8 Mean and variances (var) of the four simulated relationship categories using seven different estimators: Trio ML (TML), Wang (W), Lynch Li (LL), Lynch & Ritland (LR), Ritland (R), Queller & Goodnight (QG) and Dyad ML (DML).

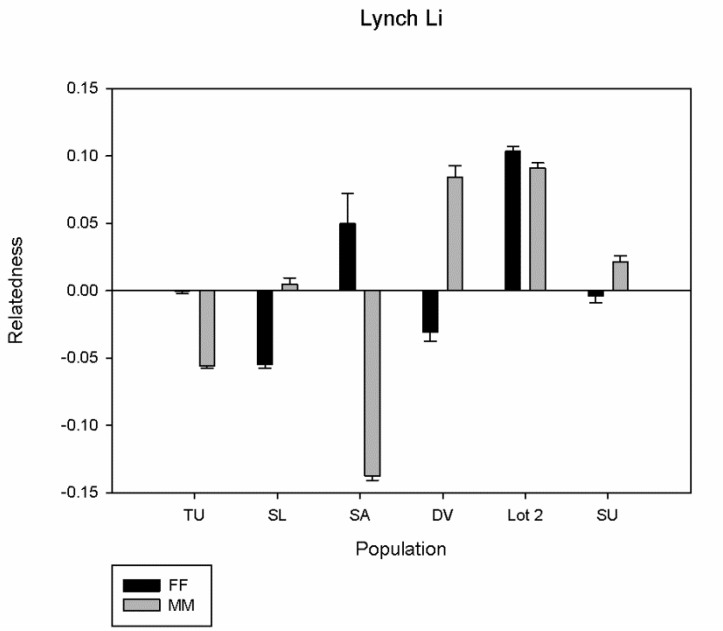
	PO (0.5)		FS (0.5)		HS (0.25)		Unrelated (0)	
Estimator	**Mean**	**Var**	**Mean**	**Var**	**Mean**	**Var**	**Mean**	**Var**
TML	0.530	0.005	0.521	0.013	0.343	0.019	0.158	0.011
W	0.486	0.008	0.484	0.022	0.251	0.034	-0.026	0.028
LL	0.489	0.010	0.481	0.022	0.251	0.036	-0.028	0.031
LR	0.623	0.017	0.622	0.020	0.443	0.026	0.238	0.028
R	0.973	0.109	0.949	0.113	0.634	0.083	0.299	0.054
QG	0.566	0.010	0.568	0.017	0.368	0.027	0.136	0.021
DML	0.559	0.006	0.564	0.013	0.381	0.017	0.192	0.013

Fig. 4.6 Relatedness estimates for female and male dyads using 4 different estimators. Average pairwise r values and standard errors are shown for female dyads (black bars) and male dyads (light gray bars) for the following sites: Tuanan (TU), Sungai Lading (SL), Sabangau (SA), Danum Valley (DV), and Suaq (SU). Estimates are based on 17 microsatellite markers. Significant differences between FF and MM dyads are indicated by * ($p<0.05$).






Queller & Goodnight
Relatedness
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
TU
SL
SA
DV
Lot 2
SU
Population
FF
MM


Lynch Li
Relatedness
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
TU
SL
SA
DV
Lot 2
SU
Population
FF
MM

Addendum: Statistical descriptor tests

Methods

We also assessed sex-biased dispersal using statistical descriptors, following the methods proposed in Goudet et al. (2002). We computed the following statistics using Fstat 2.9.3.2 (Goudet 1995): i) F_{IS}, tests for heterozygote deficiency, and is expected to be lower for the dispersing sex as they should be composed of residents as well as non residents resulting in a Wahlund effect and therefore a heterozygote deficit ; ii) F_{ST}, estimates the proportion of the total genetic variance found among populations, expected to be higher for the philopatric sex; iii) HO, the observed heterozygosity, to check for a link to inbreeding; iv) HS, the within group gene diversity, expected to be larger for the dispersing sex; v) r, the average relatedness of individuals within a population, connected to F_{ST} through $r = F_{ST} / (1- FIT)$, and expected to be higher for the philopatric sex ; vi) mean of the corrected assignment index (mAIc), calculates the probability of a multilocus genotype in a population and is expected to be higher for the philopatric sex; vii) variance of the corrected assignment index (vAIc), computes the spread from the mean for the assignment indices and is expected to be higher for the dispersing sex (Goudet *et al.* 2002). We tested significance through 10,000 randomizations and a one-tailed t-test.

Results and discussion

The statistical descriptor tests yielded higher F_{ST}, *r* and mAIc values for females as compared to males, as predicted by female philopatry and MBD, though the differences were not significant (Table 4.9). However, F_{IS} was not higher for males, as expected if they were a mixture of immigrant and non-immigrant males, although again the difference was not significant.

Table 4.9 Sex-biased dispersal statistics computed on Fstat, significance levels tested with one-sided tests and 10,000 randomizations

	Fis	Fst	r	Ho	Hs	mAIc	vAIc
F (n = 43)	0.018	0.128	0.224	0.608	0.619	0.290	12.897
M (n = 45)	-0.015	0.104	0.191	0.648	0.638	-0.277	12.938
Overall	0.001	0.118	0.211	0.628	0.629	n/a	n/a
p value	0.908	0.236	0.319	0.037	0.151	0.238	0.579

Since the sites are distributed throughout Borneo, and often separated by rivers, which are barriers to orangutan movement, it is likely that isolation by distance results in high differentiation for both males and females when examining such large spatial scales (Arora et al., 2010). Therefore it would be more suitable to check for dispersal at the scale at which an individual is physically able to disperse, that is, examining sites proximally located. Moreover, a detailed examination of the power of the statistics by Goudet et al. (2002) showed that optimal detection of sex biased dispersal occurs at intermediate dispersal rates, and high sex bias intensity. If immigrants are too rare or too frequent, they might not be detected or distinguished. Furthermore, these tests are also dependant on number of loci, levels of polymorphism, and number of individuals sampled. Thus, the power of these statistical tests is limited.

5 Discussion

5.1 Evolutionary processes of diversification and Bornean orangutan genetic diversity

This thesis has sought to understand the evolutionary processes of diversification structuring genetic diversity in Bornean orangutans. I examined several aspects: 1) the effects of dynamic environmental forces in Sundaland, including the Pleistocene glaciations, volcanic eruptions and riverine barriers, in combination with sex-biased dispersal, on historical patterns of gene flow; and 2) the effects of current sex-biased dispersal on contemporary patterns of gene flow. This latter aspect was investigated first through an in-depth case study at a site for which extensive behavioral, spatial and genetic data is available. Next, and capitalizing on the insights from the case study, relatedness and dispersal across sites were compared. In the following sections I summarize the findings of these studies and discuss the implications. In the final section, I outline some perspectives for future work.

5.1.1 The effects of Pleistocene glaciations, volcanic eruptions, rivers and sex-biased dispersal on Bornean orangutans

Summary

This study, detailed in Chapter 2, capitalized on the usage of a genetic dataset that encompassed the largest number of geographical regions and natural populations throughout the range of Bornean orangutans investigated to date. My novel results, in addition to providing strong proof for a clear-cut distinction between Bornean and Sumatran orangutans, show a complex evolutionary history in Borneo that challenge previous inferences of a smooth uninterrupted dispersal throughout the island. Analyses of the mtDNA revealed an unexpectedly recent common ancestor approximately 176 ka (95% highest posterior density (HPD) 72-322 ka) for all Bornean haplotypes, contrasting with the much older coalescence between Bornean and Sumatran haplotypes around 3 ma. Furthermore, the Bornean clade displays a star-like topology in the mtDNA phylogenetic tree. Taken together, these results indicate that despite the recent land bridges between the two islands in the last glacial period, Sumatran orangutans did not at that time contribute to Bornean genetic diversity, and that Bornean orangutan populations are the result of a recent expansion throughout the island.

Coupled with evidence that other Bornean rainforest species were probably present on the island at the time of coalescence of Bornean mtDNA haplotypes (Chapter 2; Arora *et al.* 2010), the results of this study suggest that Bornean orangutans too were present, but confined within one refugium (or several nearby ones). Most probably, this was a result of a contraction of their habitat, the rainforests, through the cooling of temperatures and decrease in precipitation that occurred during the Pleistocene glaciations (Heaney 1991; Bird *et al.* 2005). These findings also support the view that the tropics, formerly viewed as “stable” habitats during the Quaternary, were not at all exempt from the climatic changes of the Pleistocene, even if such changes may have been less severe compared to locations at middle or high latitudes (Vuilleumier 1971; Pinot *et al.* 1999; Frankham 2003).

Remarkably, despite the recent expansion of Bornean orangutans, populations are characterized by high levels of differentiation, both at the mitochondrial and at the nuclear level and there is a strong geographical clustering of mtDNA haplotypes, indicative of female site fidelity. By examining the relationship between genetic and geographical diversity, I found evidence that Bornean rivers act as strong barriers to dispersal, particularly for females whose limited movements prevent them from crossing rivers upstream where the canopies on both banks touch. These results, combined with the lower geographical structuring of Y-chromosome haplotypes (Nater 2011; Nietlisbach & Kruetzen 2011) emphasize the role of male dispersal in maintaining the genetic connectivity of populations through gene flow.

The Pleistocene glaciations

The Pleistocene glaciations have played a dual role in the population history of Bornean orangutans. On the one hand, the low sea levels of glacial periods levels opened a window of opportunity for the colonization of Borneo, most probably from Sumatra. This would have been possible when the vegetation on the exposed continental shelf was suitable for the species in question. A dispersal route from southern Sumatra to Borneo via the Bangka-Bilitung-Karimata strait is congruent with phylogenetic relationships recently ascertained by Nater and colleagues (2011b) using mtDNA genic regions. In that study, we showed that Sumatran mtDNA haplotypes are not monophyletic. Surprisingly, the haplotypes of the Sumatran population of Batang Toru, south of Lake Toba, are phylogenetically closer to Bornean haplotypes than they are to those from northern Sumatra. This relationship draws a demarcation around Mount Toba, separating the northern and southern Sumatran populations. But not only that, it further suggests that the now extinct populations from southern Sumatra may have been more related to Bornean orangutans than northern Sumatran populations, lending support to the inferred dispersal route. This dispersal agrees with a scenario of vicariant divergence as opposed to separate migrations from the mainland. It would clearly be of great interest to obtain ancient samples from eradicated southern Sumatran populations in order to corroborate these phylogenetic relationships.

On the other hand, the Pleistocene glaciations seem to have led to isolation of the Sundaland islands, probably because of the absence of suitable habitat on the newly exposed landmass. Assuming an orangutan dispersal route from Sumatra to Borneo, it is not clear at present how often it was actually used, and how many colonization and extinction events took place throughout the entire Quaternary. If the conditions were not favorable across the continental shelf, there may have been few opportunities for the migration across islands. What is known, however, that only one colonization event left a genetic signature in Borneo. Whether the successful lineage currently distributed throughout the island arrived early in the Pleistocene or later, my phylogenetic findings make a stable constant history unlikely. A smooth and stable expansion should have led to some older and more diverse lineages at locations that were first reached, and some newer and less diverse lineages in areas more recently reached. However, the analyses of mtDNA diversity do not show the presence of a diversity gradient from the southwest of the island, or from any other direction for that matter (see Appendix II), thus making such a scenario untenable.

I have proposed that within Borneo, the recent radiation of orangutan populations and the star-like mtDNA phylogeny suggest that the surviving lineage of orangutans was

confined to a refugium or several refugia, while other lineages probably went extinct, during a glacial period. One argument raised against this scenario is that the same genetic signature on the mtDNA could be left by another process: a selective sweep or rapid fixation of a new advantageous allele, with a transient excess of rare polymorphisms (Handley *et al.* 2007; Pilkington *et al.* 2008). Such a sweep would imply that current mtDNA diversity levels and coalescence time do not mirror demographic processes but the time since the last sweep. But this possibility seems unlikely. Analyses of the mtDNA analyses show a very tight geographical structuring of haplotypes, in agreement with behavioral evidence for female philopatry. Furthermore, populations are highly differentiated for both autosomal and mtDNA markers, with dispersal limited by geographical barriers such as rivers. Under the observed limited gene flow, primarily male-mediated, it would be hard to envision the rapid spread of an advantageous mtDNA variant throughout all populations in Borneo. Nonetheless, it is of interest to contrast the patterns in different markers, since the effects of a selective sweep would be confined to a single locus, but a population bottleneck followed by expansion should produce the same pattern on other markers too. To date, a study using the Bornean and Sumatran orangutan genomes and testing two demographic models found support for a decline in the effective population size of Bornean but not Sumatran orangutans starting around 400 ka (Locke *et al.* 2011). Usage of a larger sample size incorporating individuals from various sites, and the testing of more complex scenarios are, however, necessary for more robust conclusions.

Biogeographic studies of several species including primates, plants and insects support the existence of refugia in Sundaland, reservoirs sustaining biotic diversity during adverse environmental conditions (Barkman & Simpson 2001; Gathorne-Hardy *et al.* 2002; Cannon & Manos 2003; Meijaard & Groves 2004; Quek *et al.* 2007; Ziegler *et al.* 2007; Jalil *et al.* 2008). It is of course tempting to speculate on the location of a suitable refugium or refugia in Borneo. Mixed results on the reconstruction of the Bornean paleo-environment have been obtained by various studies, depending on the type and location of the samples examined. Such equivocal signals could partly be explained through spatial variation in micro-climates, associated with longitudinal and altitudinal differences (Louys & Meijaard 2010). From northeastern Borneo, there is evidence for tropical rainforests persisting during the last two glaciations, so this region is a good candidate for hosting orangutans and other species during predominantly unfavorable conditions (Visser *et al.* 2004; Louys & Meijaard 2010).

The isolation of the island of Borneo during glacial periods coupled with the probable population contraction within a glacial refugium most probably promoted high genetic divergence between Bornean and Sumatran orangutans. Among some of the most notable differences between Bornean and Sumatran genomes are chromosomal rearrangements, and given the link proposed between such rearrangements and speciation (Wilson *et al.* 1975; Coyne & Orr 2004a; Kirkpatrick & Barton 2006), it is tantalizing to speculate on their role in the orangutans. There are several of these karyotypic differences distinguishing Bornean and Sumatran individuals, including a pericentric inversion in chromosome 2 (Seuanez *et al.* 1979), an inversion in chromosome 3 (Locke *et al.* 2011) and an inverted and satellited Y chromosome (Schempp *et al.* 1993a). One possibility is that such rearrangements went to fixation in a small population such as in a founding event or in the proposed Bornean refugium, and are partly responsible for the reduced hybrid fitness of Bornean and Sumatran orangutans found in a captive study (Cocks 2007). Assuming this role, these rearrangements would act as postzygotic isolating

mechanisms in the event of secondary contact. Alternatively, it is also possible that the gene arrangements got fixed through selection if they suppressed recombination of locally adapted genes (Kirkpatrick & Barton 2006). This possibility warrants a closer examination of the genes captured by inversions and the selective forces acting upon them. In any case, another chromosomal rearrangement has recently been found in chromosome 12, but in contrast to the others, the two variants are found in both Bornean and Sumatran orangutans. This puzzling polymorphism was proposed to have arisen before the split of the two species (Locke *et al.* 2011) and would thus represent retention of variation. Clearly, chromosomal rearrangements present a very interesting field of study that merits further work to establish the extent to which they contribute to speciation. Some clues as to how and when chromosomal differences arose may be provided by the southern Sumatran orangutans from Batang Toru, whose mtDNA haplotypes are phylogenetically closer to those of Borneo than northern Sumatra. It would be particularly interesting to examine whether these individuals display the rearrangements specific to one island or another, or both.

Aside from the oscillating glacial and interglacial changes, volcanic eruptions have also been prominent in the history of Sundaland (Chesner & Luhr 2010). Strikingly, there is no evidence that the Toba super-eruption approximately 74 ka led to population extinctions in orangutans. The coalescence date for the Bornean mtDNA haplotypes does not conclusively rule out the Toba eruption as a cause for the extinction of lineages and a population contraction. But the fact that much older Sumatran mtDNA lineages are found around Mount Toba makes it very unlikely, unless the climatic effect was vastly different in Sumatra compared to Borneo (Nater *et al.* 2011b).

Comparison with other species

High genetic differentiation between the Bornean and Sumatran orangutan species arose at least partly due to insular isolation: both unsuitable habitat during glacial periods and high sea levels during interglacial periods impeded gene flow. Insular isolation also accounts for the allopatric divergence of Bornean and Sumatran populations of species including gibbons, macaques, clouded leopards, and rhinoceros, as well as the fascinating number of endemic forest mammals in Borneo (Wilting *et al.* 2007; Ziegler *et al.* 2007; Earl of 2010; Thinh *et al.* 2010). Perhaps one of the most striking outcomes of insular isolation is the phenomenon of dwarfism or reduction in body size that numerous species experienced during the Pleistocene in various regions of the world including Borneo, where large mammals are smaller than on the neighboring Sunda islands (Meiri *et al.* 2008). This phenomenon has also been proposed to account for the evolution of *Homo floresiensis*, the small-bodied hominin from the island of Flores, following divergence from the ancestral *Homo erectus* during a period of ca 800 ka (Brown *et al.* 2004). It is still debated, however, whether *Homo floresiensis* really diverged from *Homo erectus* or whether it represents a different lineage altogether that might have been subject to some reduction in size (Jungers *et al.* 2009; Morwood & Jungers 2009). The effects of insular isolation are remarkable, and further comparative studies are expected to shed light on the full extent of genetic and phenotypic differentiation it can produce.

The climatic and vegetational fluctuations of the Pleistocene did not only isolate islands but as I have previously discussed, also led to changes in the distribution of suitable habitat within islands. For Bornean orangutans, although there is some uncertainty as to the precise time to coalescence of the mtDNA haplotypes, the mean falls within the

penultimate glacial period, also referred to as the Marine Isotope Stage 6 (MI6), between 130-190 ka, suggesting that this may have been a key event in their history. This was a particularly harsh glacial period, with the lowest temperatures of the entire Quaternary. A comparison of the phylogeography of other taxa can provide us with clues to the importance of this particular historical event.

So far, it seems that the Bornean gibbons *Hylobates muelleri* distributed to the north, south and east of the island, did not suffer dramatic impacts from the last two glacial periods. The older coalescence of their mtDNA haplotypes around 1.78 ma (95% confidence interval between 1.33 - 2.25) would suggest that older lineages survived in Borneo (Thinh *et al.* 2010). Several reasons might account for this difference with orangutans. Firstly, their social organization differs markedly from that of the orangutans, with both female and male dispersal. In fact, in the closely related southern Sumatran siamang gibbons (*Symphalangus syndactylus)*, the absence of geographical structuring and higher mtDNA diversity of females compared to males indicate that females travel longer distances (Lappan 2007). It is plausible then that *Hylobates* has a similar pattern of dispersal. In any case, female dispersal prevents the geographical clustering of mtDNA diversity, so the loss of some populations would not have reduced their mtDNA levels as drastically. Second, gibbons might be able to survive in small forest patches at higher densities, thus allowing them to maintain a higher census and effective population size compared to orangutans. Nevertheless, a comprehensive population genetic study of Bornean gibbons as well as other Bornean large-bodied mammals, is still lacking, so further work is required before we can understand how historical processes shaped their current distribution and diversity.

As for the hominins living in Sundaland at that time, it is only through the fossil record that we can ascertain the effects of the changing environment on their distribution. And indeed, faunal and stone tool remains from the Indonesian cave of Liang Bua indicate that the occupational intensity of *Homo floresiensis* fluctuated from 100-17 ka, peaking at wetter phases. Drier periods, by contrast, might have driven these hominins either to the open-air or other locations. In either case, these findings demonstrate that the Flores hominins adapted to changes in precipitation and vegetation cover by shifting to their preferred locations (Westaway *et al.* 2009).

Meanwhile in Africa the harsh conditions of the penultimate glaciation that probably confined Bornean orangutans to a refugium also appear to have affected our ancestors through a major bottleneck around the time of origin of modern humans (Fagundes *et al.* 2007), with estimates of nuclear and mtDNA coalescence at 144 ka (95% CI 104 - 186 ka; Fagundes *et al.* 2007), 194.3 ±32.5 ka (Gonder *et al.* 2007), and 203±12.6 ka (Behar *et al.* 2008). But the penultimate glaciation might not only have produced a bottleneck: it may have also been accompanied by notable behavioral changes. In fact, the elevated sea cave in South Africa from ~164 ka (±12 ka) provides the earliest evidence for modern human habitat and diet expansion to occupy coastal areas and include marine resources such as shellfish as food. This shift is interpreted as a flexible response to MI6, one of the coldest driest glacial periods (Marean *et al.* 2007; Marean 2010). It is possible too that this shift is associated with the population expansion that was detected using non-coding regions of the X chromosome starting around 160 ka (Kaessmann *et al.* 2001).

One other event that occurred during the Pleistocene, shortly before the start of a cold stadial, is the Toba super-eruption, considered to be the largest eruption of the entire

Quaternary (Ninkovich *et al.* 1978). Much controversy surrounds the event, and several researchers have argued that the nuclear volcanic winter that ensued led to a severe human bottleneck in Africa with small isolated populations surviving in refugia, as well as the extinction of many large mammals in Southeast Asia (Ambrose 1998; Williams *et al.* 2009). However, old orangutan lineages in northern Sumatra have survived at locations in geographical proximity to Mount Toba, arguing against the devastating consequences proposed for this super-eruption (Nater *et al.* 2011b). This finding also draws attention to the volcanic eruption thought to account for the extinction of *Homo floresiensis* ca. 17 ka, which might need further careful examination.

Anthropogenic and climatic effects: conservation implications

One issue that has not yet been discussed is the consequence of the arrival of modern humans in Southeast Asia. It is hypothesized that modern humans reached Indonesia sometime between 65-45 ka (Macaulay *et al.* 2005; Mellars 2006), and there is evidence for their activity at the Niah cave in Borneo since around 40 ka (Barker *et al.* 2007). At this cave faunal and tool remains from the late Pleistocene and early Holocene indicate that modern humans hunted orangutans and that these probably survived in the surrounding areas up until the last millennium (Barker *et al.* 2007; Earl of 2010). To what extent hunting may have reduced the number of orangutans is unknown. But analyses of mtDNA diversity of the Niah orangutan samples would provide us with some clues on whether this diversity is still found today in neighboring areas or is completely extinct. In Java, by contrast, the disappearance of orangutans and other arboreal species from the Holocene fauna is associated with a change to drier and more seasonal climates, and not to human intervention (van den Bergh *et al.* 2001; Earl of 2010).

Interestingly, some orangutan population expansions may have been very recent, as suggested by two sites I have examined. Both Danum Valley (DV) and Gunung Palung (GP) are located in drier regions composed of lowland dipterocarp rainforests, whereas many of the other sites are in peat swamps. Moreover, both DV and GP have reduced mtDNA diversity that seems to be a subset of another population. For instance, only two mtDNA haplotypes have been found at DV, both of which are shared with South Kinabatangan (SK). These two populations are only about 90 km apart and not physically separated by any barriers. GP shares its only mtDNA haplotype with Sabangau (SA), from which it is, by contrast, geographically separated by a few rivers. From the mtDNA data, we may speculate that these pairs of populations are phylogenetically closely related and that DV and GP arose rather recently. For GP, given the separation from SA, there may have been an ancestral population at an intermediate location, providing an interesting prospect for future sampling. When exactly these possible expansions took place should also be of particular relevance to understanding more recent orangutan population dynamics.

Contrasting with these expansions, a dramatic decline in population size has been reported for the Kinabatangan orangutans occurring within the last few centuries. The dating of this population collapse points to recent human induced-deforestation as possibly an even more critical danger than past hunting (Goossens *et al.* 2006a). At a Borneo-wide scale, the last few decades in particular have witnessed severe habitat fragmentation that impedes male-biased dispersal, leading not only to potential inbreeding, but also to greater population genetic differentiation that would have severe consequences on the loss of genetic diversity should some populations go extinct.

In sum, these phylogeographic and population genetic structure findings show that Bornean orangutans were highly susceptible to environmental and geographical forces in the past, and are thus likely to be extremely vulnerable to climate and habitat changes in the future.

5.1.2 Contemporary patterns of sex-biased dispersal in a population of Bornean orangutans

Summary

In order to understand contemporary patterns of gene flow determined by sex-biased dispersal, I carried out a fine-scale genetic investigation at the long-term Bornean site of Tuanan (Central Kalimantan, Indonesia) as detailed in Chapter 3. At this site, established by the Anthropological Institute and Museum, simultaneous genetic sampling and behavioral observation of individuals permitted the generation of the largest database with combined data on space use, individual behavior, and genetic relationships. Through the examination of mtDNA diversity patterns, which reflect maternal ancestries, and the reconstruction of genealogical relationships, a solid pattern of female philopatry and male-biased dispersal emerged. The analyses in this study revealed the presence of matrilineal clusters, that is, females with highly overlapping home ranges that were closely related as mothers, daughters and/or sisters. The presence of kin structures among females paves the way for further analyses of individual associations, and for possible nepotistic cooperation in orangutans. As for the males, the absence of maternal relatives in the study area, and the highly divergent mtDNA haplotypes of some of these confirmed that they are indeed the dispersing sex. Furthermore, I rarely found maternal or paternal relatives among the males. Although some males did share their mtDNA haplotype, and thus may be maternally related, the limited associations among orangutan males (Delgado & Van Schaik 2000) make the possibility of parallel dispersal unlikely.

In addition to elucidating the relatedness patterns among individuals in Tuanan, an integrated approach provided the first hints to some key issues in the ability to unravel such patterns. First, the importance of spatial scale in sampling became evident. Through a parentage-based pedigree reconstruction, I determined the presence of three female matrilines. Two of these comprised females whose home ranges were mainly within the study area, and for these I was able to disentangle complete genealogical relationships. The females of the third matriline, however, ranged partly in the periphery and for these I could only establish a few relationships, suggesting that their relatives were not sampled because they were not within the study area. These findings highlight the caveats of the locational-based sampling approaches applied to non-gregarious mammals compared to the group-based sampling of gregarious taxa. They further emphasize the importance of incorporating space use information to assess whether the genetic and social "neighborhood" of the study individuals has been sampled, as discussed later on.

Second, I also noted the key role of life history traits in determining relatedness among individuals. In species with slow life histories and low reproductive output such as orangutans and other great apes, the number of highly related dyads among philopatric individuals is limited, even when site fidelity is extreme. Furthermore, while high reproductive skew increases levels of coancestry within the philopatric sex, low reproductive skew has the opposite effect, and leads to lower relatedness among philopatric individuals, especially in systems with male philopatry and female-biased

dispersal (Lukas *et al.* 2005). Such traits are expected to constrain the opportunities for kin selection to operate.

Third, my findings illustrated the critical importance of substructuring: in orangutans, several matrilineal clusters may overlap in their home ranges. While each cluster comprises closely related maternal dyads, females across clusters need not be related. Consequently, a high number of unrelated dyads might still be found among the philopatric sex. This stacking of matrilineal clusters may also be present in other non-gregarious species, but might go detected unless genealogical relationships are ascertained.

With these findings in mind, I explored other long-term study sites to determine general patterns of sex-biased dispersal in orangutans. I also sought to elucidate whether the discrepant results from previous genetic studies of instantaneous or one-generation dispersal stem from intra- or inter-specific variation, or might have been due to methodological issues. For this meta-population investigation, which constitutes Chapter 4, I included several sites in Borneo and one in Sumatra. The results across sites were generally consistent with a model of female philopatry and male-biased dispersal in orangutans. I found various sex-specific haplotypes at several sites, pointing to the different maternal ancestries of females compared to males. The parentage-based pedigree reconstruction revealed the presence at all sites of maternally related female dyads, while at most sites relatives among males were significantly less frequent. Although mothers were assigned to a few males, these were generally unflanged and young, suggesting that at the time of sampling they had yet not dispersed. At one site, habitat fragmentation might account for reduced male mobility. Moreover, I tested whether the average pairwise relatedness (r) of females was higher than for males, and found a tendency at most sites, although it was not significant. Critically, when I took maternally ancestry into account, r estimates for females within a cluster were consistently higher than when calculated by pooling together all females from a site, showing the importance of female substructuring.

Based on these findings, I delved further into the issues that determine our ability to detect sex-biased dispersal in non-gregarious species such as orangutans. As revealed by the pedigree reconstruction, the slow life history and low reproductive output of orangutans results in small sets of closely related individuals. Detecting these depends largely on the sampling regime employed, and particularly, on the size of the sampling area relevant to home range size of individuals. As observed in the fine-scale study of Tuanan, it is important to encompass the entire home ranges of females to incorporate as many "neighbors" as possible, because these probably constitute the genetic and social network of a female.

I also found that *r* estimates are poor indicators of relatedness in orangutans for a number of reasons. First, marker quality and quantity might be insufficient to disentangle relationship categories such as half-sib and unrelated dyads. This was the case in my study and probably also in other investigations, although assessments are infrequently conducted or reported. Second, particularly in species for which sample sizes are small, as in orangutans, r estimates can vary widely across estimators, making it difficult to draw reliable conclusions. Third, as illustrated in the case study of Tuanan, female orangutans form matrilineal clusters with overlapping home ranges, and while females within a cluster might comprise "families" of mothers, daughters and maternal sisters,

females between clusters might not be related. Such substructuring reduces the power of r estimates to detect differences between the sexes. Furthermore, biases may result not only from small sample sizes but also as mentioned before from an opportunistic sampling regime that does not take into account the full "neighborhood" of a female. These factors probably played a role in the discrepancies of genetic tests of sex-biased dispersal in orangutans found to date, and I propose that they might also be important in other non-gregarious species. Consequently, and based on these outcomes, it may be important to revise some of the predictions for estimates of relatedness in these species.

Most importantly, these findings highlight the critical need to integrate various lines of evidence in order to fully disentangle patterns of relatedness and dispersal: the combined use of spatial and behavioral information, as well as various genetic leads to a more complete and accurate picture of sex-biased dispersal.

Comparison with other great apes

Taken together, the examination of dispersal in orangutans strongly supports behavioral observations of female philopatry and male dispersal. The natal site fidelity of orangutan females, and the associated female kin structures it gives rise to, contrast with routine female dispersal in other extant great apes (Eriksson *et al.* 2006; Douadi *et al.* 2007; Langergraber *et al.* 2007). Assuming the ancestor of the great apes was also characterized by the predominant pattern in mammals of female philopatry, the variable social organization of the African great apes and humans would be derived.

But what led to this change or series of changes in the African great apes and humans? Several evolutionary explanations have been put forth to explain why females emigrate from their natal groups. In chimpanzees, for instance, females forage alone to avoid the high costs of space and food competition while males defend feeding territories through patrolling behavior (Mitani *et al.* 2002). Thus, males might reap high rewards through long-term stable associations with other males, attaining indirect fitness benefits if they are related (Mitani 2009). The advantages of philopatry for males, together with their long tenure, which may surpass a female's age at first reproduction, might compel females to migrate to avoid breeding with close male relatives (Clutton-Brock 1989). In orangutans, although females also engage in solitary foraging, the high energetic costs of an arboreal lifestyle might prevent male association to defend access to females (Visser *et al.* 2004)

Inbreeding avoidance might also explain the differential dispersal distances of females and males in western and mountain gorillas. Male tenure in breeding groups is very long, but females reaching sexual maturity can avoid inbreeding by transferring to neighboring groups (Clutton-Brock 1989; Douadi *et al.* 2007). Males by contrast, face high mating competition but cannot as easily immigrate into other neighboring groups, and might thus need to travel larger distances (Douadi *et al.* 2007). Another non-exclusive explanation for mountain gorillas is that females transfer from one-male to multi-male groups because these confer greater infanticide protection, which is in fact the reason why females join males in a group in the first place (Van Schaik & Kappeler 1997; Harcourt & Greenberg 2001; Robbins *et al.* 2009).

In humans, female dispersal is also common, particularly in the patrilocal societies that arose after the agricultural revolution (Lawson Handley & Perrin 2007), in which males

can defend resources. But more recently it has become clear from studies of hunter-gatherers that humans actually display, in contrast to most of the other great apes, a remarkable diversity in social organization. In these societies, deemed representative of most of our evolutionary history, both female and male dispersal occur. Also striking in these societies is that, while brother-sister pairs occur frequently in residential units, most pairs are unrelated or only distantly related (Hill *et al.* 2011). Thus a high degree of flexibility characterizes human social systems.

To resolve whether this flexibility is a hallmark of modern humans or was also found in other closely related species, we need to turn to the now extinct hominins. But so far most reconstructions for these species are highly speculative. Male patrilocality was suggested for Neanderthals given findings of shared maternal ancestry among the males of a social group (Lalueza-Fox *et al.* 2011). This proposition is, however, disputed because maternal ancestry does not necessarily imply high relatedness among males (Vigilant & Langergraber 2011). In early hominins such as Australopithecines, the non-local characteristics of many of the smaller teeth that presumably belonged to females were also interpreted as evidence for greater female dispersal (Copeland *et al.* 2011). Nevertheless, the uncertainty surrounding the origin of the teeth does not allow firm conclusions here either. Consequently, we cannot determine whether other hominins also had flexible co-residence patterns.

Overall, it seems that there are greater benefits to female dispersal in the African great apes and humans, compared to orangutans where male alliance formation is completely lacking. Nonetheless, and quite strikingly, female emigration does not preclude strong social bonds among females, and not only among humans. In western and mountain gorillas, females sometimes disperse to groups where they have relatives, and can thus still establish same-sex nepotistic associations even when they are not philopatric (Bradley *et al.* 2007; Guschanski *et al.* 2008). In chimpanzees and bonobos, the importance of female associations is underscored by the long-term social bonds between females that occur even in the absence of relatedness (Gerloff *et al.* 1999; Langergraber *et al.* 2009). So in orangutans, where female kin structures are prevalent, it will be of great interest to establish whether opportunities for nepotistic cooperation also influence philopatry in the studies already underway (Van Noordwijk 2011b).

5.1.3 Conclusions: orangutans among great apes

To recapitulate the findings of this thesis, I have shown that current orangutan populations are generally characterized by extreme female philopatry and male-biased dispersal, and that some of the discrepant genetic results examining current dispersal patterns may stem from methodological issues. My finding also demonstrated contemporary male-mediated gene flow that agrees with historical patterns inferred from orangutan phylogeography. Furthermore, I argued that the distribution and population genetic structure of Bornean orangutans have been strongly shaped by the climatic and vegetational fluctuations of the Pleistocene, which resulted in the repeated connection and isolation between Borneo and other islands. In particular, one of these fluctuations may have been responsible for a contraction of Bornean orangutans within a refugium, which should have led to greater and faster genetic divergence from other orangutans. The probable extinctions of other populations in Borneo at the time of the bottleneck only highlight the vulnerability of orangutans.

Isolation in Pleistocene refugia is not unique to orangutans among the great apes. Genetic studies show that gorillas and humans were probably also constrained in refugia (Jensen-Seaman *et al.* 2001; Anthony *et al.* 2007). In particular, gorillas, which like orangutans show great habitat specificity, may have been especially affected by the Pleistocene changes (Jensen-Seaman *et al.* 2001). However, the extreme sex-biased dispersal of orangutans has probably resulted in far greater structuring of genetic diversity through the high levels of coancestry among philopatric females. Such high structuring and population differentiation exacerbates the risks of loss of genetic diversity through anthropogenic habitat fragmentation and future climatic change, calling for urgent action towards orangutan conservation.

5.2 Future work

The results presented herein open the way for several avenues of research. First, we can refine the phylogeography of Bornean orangutans by filling the distributional gaps through sampling of new regions. This should aid in several ways. Attaining greater detail enables more precise identification of the geographical origin of rehabilitants for example. Moreover, sampling other areas can also be of special relevance in determining the characteristics of spatial expansions. I have shown that Gunung Palung (GP) and Danum Valley (DV), two populations in dry regions which display extremely low mtDNA diversity levels, appeared to be subsets of other populations from peat swamp habitats. Further comparisons of other populations should provide clues to whether expansions from rich to poor habitats in the recent past have been common. Additionally, a more complete portrayal of genetic diversity throughout the island could help unravel the pattern of mutational distances in mtDNA haplotypes across sites. For instance, it is unclear why the pair TU/SL has more pairwise differences than TU/SK, given that the former pair is in greater geographical proximity. Naturally, the presence of geographical "bridges" and barriers should have played a major role in population histories, deserving greater focus. Such detailed investigations might also help pinpoint the location of the proposed refugium (or closely situated refugia).

Second, the extension of sampling to the micro-geographic scale, that is, in and around study sites, offers some interesting possibilities, despite the obvious logistical and financial obstacles. Bornean orangutan long-term study sites are separated by large distances around the rivers, and it is thus difficult to identify the sources of immigrants or estimate how far they travel with any precision. Yet this would be of use in determining the spatial characteristics of gene flow. Sampling regionally would allow assessment of the rate of sex-biased dispersal, for instance by determining whether some females do in fact disperse. If they do, we might ask what factors promote such behavior, including food distribution and population density. Moreover, it would be valuable to ascertain whether female long-distance dispersal, if found to occur, is sufficient to lead to new stable populations. These investigations would allow not only a better understanding on the flexibility underlying dispersal in orangutans, but also, from a conservation perspective, more powerful predictions on the consequences of further environmental change. Additionally, they may enable us to explore some puzzling questions, such as for instance why DV and SK, despite sharing of mtDNA haplotypes, and not having a riverine barrier between them, are significantly differentiated at the autosomal level.

These are only a few of a number of possibilities in which orangutans can help us tackle some of the most exciting and challenging questions in evolutionary biology. Their

especially unusual features, as well as their close relationship to humans, render these species a rich and promising field for further research.

6 Co-authored publication and manuscript abstracts

6.1 Sex-biased Dispersal and Volcanic Activities Shaped Phylogeographic Patterns of Extant Orangutans (genus: *Pongo*)

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Abstract

The Southeast Asian Sunda archipelago harbors a rich biodiversity with a substantial proportion of endemic species. The evolutionary history of these species has been drastically influenced by environmental forces, such as fluctuating sea levels, climatic changes, and severe volcanic activities. Orangutans (genus: Pongo), the only Asian great apes, are well suited to study the relative impact of these forces due to their well-documented behavioral ecology, strict habitat requirements, and exceptionally slow life history. We investigated the phylogeographic patterns and evolutionary history of orangutans in the light of the complex geological and climatic history of the Sunda archipelago. Our study is based on the most extensive genetic sampling to date, covering the entire range of extant orangutan populations. Using data from three mitochondrial DNA (mtDNA) genes from 112 wild orangutans, we show that Sumatran orangutans, *Pongo abelii*, are paraphyletic with respect to Bornean orangutans (P. pygmaeus), the only other currently recognized species within this genus. The deepest split in the mtDNA phylogeny of orangutans occurs across the Toba caldera in northern Sumatra and, not as expected, between both islands. Until the recent past, the Toba region has experienced extensive volcanic activity, which has shaped the current phylogeographic patterns. Like their Bornean counterparts, Sumatran orangutans exhibit a strong, yet previously

undocumented structuring into four geographical clusters. However, with 3.50 Ma, the Sumatran haplotypes have a much older coalescence than their Bornean counterparts (178 kya). In sharp contrast to the mtDNA data, 18 Y-chromosomal polymorphisms show a much more recent coalescence within Sumatra compared with Borneo. Moreover, the deep geographic structure evident in mtDNA is not reflected in the male population history, strongly suggesting male-biased dispersal. We conclude that volcanic activities have played an important role in the evolutionary history of orangutans and potentially of many other forest-dwelling Sundaland species. Furthermore, we demonstrate that a strong sex bias in dispersal can lead to conflicting patterns in uniparentally inherited markers even at a genus-wide scale, highlighting the need for a combined usage of maternally and paternally inherited marker systems in phylogenetic studies.

6.2 A multiplex-system to target 16 male-specific and 15 autosomal genetic markers for orangutans (genus: *Pongo*)

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Abstract

Genetic studies of dispersal on local spatial and short temporal scales require a large number of autosomal microsatellites. However, the study of dispersal over large spatial scales and the resolution of deep evolutionary histories require marker systems that are preferentially inherited through the male or female line. Addressing such questions in endangered orangutans (genus: *Pongo*) bears significant relevance to species conservation, as habitat destruction and fragmentation pose a significant threat to the whole genus. Here, we report 16 male-specific markers (nine human-derived microsatellites, six single nucleotide and one insertion-deletion polymorphisms), and 15 novel *Pongo*-derived autosomal microsatellite loci. All 31 markers can be amplified in four multiplex polymerase chain reactions even in DNA derived from faecal material. The markers can be applied to studying a wide range of important questions in this genus, such as conservation genetics, social structure, phylogeny and phylogeography.

6.3 Female philopatry and its social benefits among Bornean orangutans

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In preparation

Abstract

Female philopatry in mammals is generally associated with ecological and sometimes with social benefits, and often with dispersal by males. Previous studies on dispersal patterns of orangutans, largely non-gregarious Asian great apes, have yielded conflicting results. Based on mitochondrial and nuclear DNA analyses on faecal samples of 15 females and 31 males from a population of Bornean orangutans (*Pongo pygmaeus wurmbii*) in Tuanan subject to long-term study, we provide both genetic and behavioural evidence for male dispersal and female philopatry. Although adult female dyads that are maternally related may show similar home-range overlap as some unrelated dyads, females spend much more time in association with known maternal relatives than with other females. While in association, offspring of maternally related females frequently engage in social play, whereas this is actively prevented during encounters between unrelated mothers. Thus, Bornean orangutan females derive not only ecological but also social benefits from philopatry. Even when rarely expressed, such benefits may be more common for solitarily foraging species than currently documented, thus explaining the ubiquity of female philopatry in such species. The results also show that female dispersal and male philopatry do not characterize all great apes, casting doubt on the idea that such tendencies in humans reflect a deeply constrained shared derived trait of the whole great-ape lineage.

6.4 Call cultures in orangutans?

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Abstract

During the last decade, several studies have suggested the presence of cultures in our closest relatives, the great apes, arguing that human cumulative culture presumably evolved from such a foundation. These studies focused on conspicuous behaviours, such as tool use and social signals, and showed rich geographic variation, which could not be attributed to known ecological or genetic differences between populations. Although within call type geographic variation has been reported for orangutans, here we show the first evidence for geographic variation among orangutan populations in discrete call types. In exactly the same behavioural context, individuals in different wild populations

customarily emit qualitatively different calls or no calls at all. By comparing patterns in call-type and genetic similarity for five different populations, we suggest that the variation found here is not explained by genetic differences alone, while a simple ecological explanation is also not supported. Thus although we do not show social learning directly, these results suggest the potential of 'call cultures' and imply that wild orangutans possess the ability to invent arbitrary calls, which subsequently spread through social learning (as recently demonstrated in captive orangutans). We conclude that a more elaborate repertoire of such arbitrary calls, perhaps coupled with a similar repertoire of gestures, could be within the cognitive reach of great apes, and could have formed the kind of communication system upon natural selection could build the vocalized version, i.e. human speech.

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8 Appendix I

Population Expansions

Neutrality tests

Tajima's D (Tajima 1989) examines the difference in the population parameter θ as estimated from nucleotide diversity and from the number of segregating sites. If the difference is negative due to low frequency mutants, the population is inferred to be growing. Tajimas' D is based on the infinite sites model.

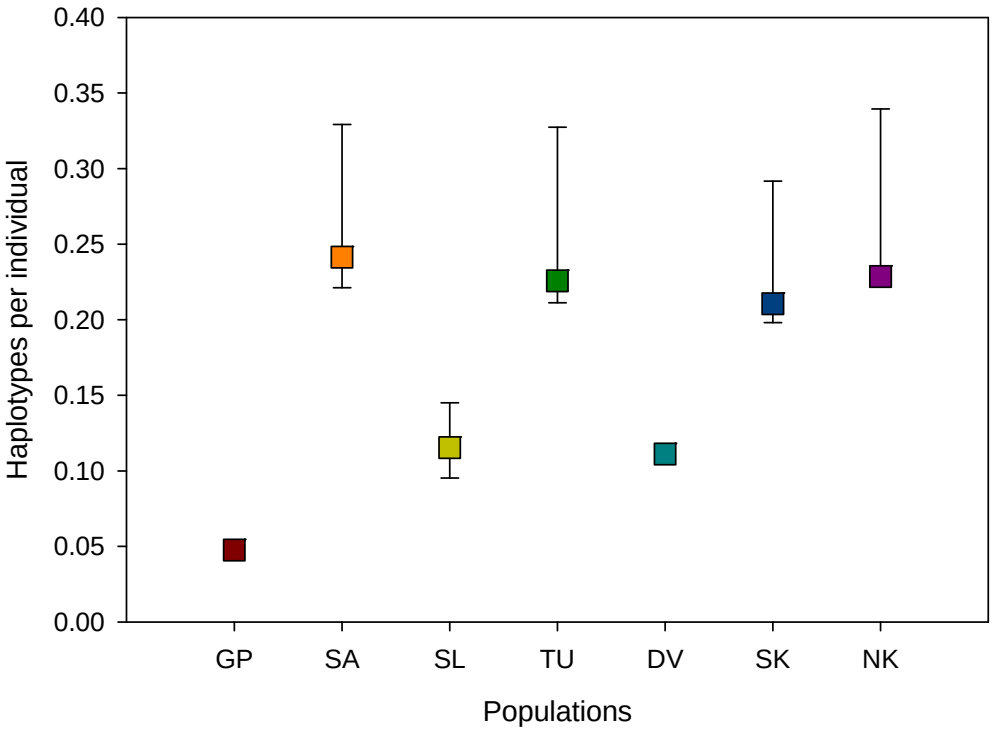
Fu's Fs (Fu 1997), which is also based on the infinite sites model, examines the number of alleles (K) expected under neutral evolution taking the number of pairwise differences into account. Again if there is an excess of rare alleles, negative Fs values will indicate population expansion.

Fu and Li's D* (Fu & Li 1993) is based on the difference between the number of singletons and the average number of nucleotide differences between sequences.

9 Appendix II

MtDNA Diversity Analyses

The figure shows the mean number of haplotypes per individual per site with 95% confidence intervals. These were calculated using an ordinary nonparametric bootstrap with 10,000 replicates to estimate the lower and upper confidence intervals for each population. For GP and DV no confidence intervals could be estimated as all bootstrap replicates had equal values (1 and 2 estimated haplotypes respectively).



10 Appendix III

Reprinted publications

Effects of Pleistocene glaciations and rivers on the population structure of Bornean orangutans (*Pongo pygmaeus*)

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Sundaland, a tropical hotspot of biodiversity comprising Borneo and Sumatra among other islands, the Malay Peninsula, and a shallow sea, has been subject to dramatic environmental processes. Thus, it presents an ideal opportunity to investigate the role of environmental mechanisms in shaping species distribution and diversity. We investigated the population structure and underlying mechanisms of an insular endemic, the Bornean orangutan (*Pongo pygmaeus*). Phylogenetic reconstructions based on mtDNA sequences from 211 wild orangutans covering the entire range of the species indicate an unexpectedly recent common ancestor of Bornean orangutans 176 ka (95% highest posterior density, 72–322 ka), pointing to a Pleistocene refugium. High mtDNA differentiation among populations and rare haplotype sharing is consistent with a pattern of strong female philopatry. This is corroborated by isolation by distance tests, which show a significant correlation between mtDNA divergence and distance and a strong effect of rivers as barriers for female movement. Both frequency-based and Bayesian clustering analyses using as many as 25 nuclear microsatellite loci revealed a significant separation among all populations, as well as a small degree of male-mediated gene flow. This study highlights the unique effects of environmental and biological features on the evolutionary history of Bornean orangutans, a highly endangered species particularly vulnerable to future climate and anthropogenic change as an insular endemic.

Asian great ape | genetic structure | radiation | geographical barriers | sociobehavioral barriers

Environmental mechanisms are some of the most important forces affecting the evolutionary history and current distribution of species. Such mechanisms have been invoked to explain genetic structure in many temperate European and North American species but with little focus on hotspots of biodiversity and endemism in the tropics (1), where the forces underlying patterns of genetic diversity and differentiation are especially intriguing.

The tropical Asian hotspot of Sundaland is remarkable in that it has been subject to dramatic geological and environmental changes (2, 3). This now partly submerged continental shelf encompasses the Malaysian peninsula, the islands of Borneo, Sumatra, Java, and possibly Palawan (2). It is a historically dynamic tectonic area that underwent notable landmass configuration changes (3). More recently, it has been severely affected by the Pleistocene climatic oscillations (4) of the Quaternary. Changes in sea levels resulted in the cyclical exposure of the continental shelf and the formation of land bridges between the islands (4, 5), allowing for species interchange with subsequent isolation (6). Moreover, climatic fluctuations were accompanied by vegetation changes (2, 7, 8), with shifts in the range and elevational distribution of rainforests. Thus, these changes led to habitat expansions or contractions, leading to new openings or barriers to gene flow. The Pleistocene was further punctuated by intense regional climatic and habitat changes through extraordinary volcanic eruptions, especially of Mount Toba (9, 10). Finally, Sundaland contains many interesting topographical features, including rivers, lakes, and mountains (5, 11, 12), that may have acted as barriers to dispersal for a number of species, adding yet another potential allopatric force.

The roles of these environmental forces in driving biotic diversity and endemism remain underexplored, particularly in Borneo, the world's second largest tropical island as well as the easternmost Sunda region abutting the Wallace line (13, 14). Its unusually high species endemism (14–16) suggests a combination of specialized ecological niches, refugia formation, and long periods of isolation.

Among the species endemic to the island are the Bornean orangutans (*Pongo pygmaeus*). This rainforest canopy-bound species with an unusually slow life history is characterized by a rich spectrum of genetic, morphological, and cultural variation (17–19). Fossils indicate a much wider distribution of orangutans during the Pleistocene extending from Southern China and Vietnam to Java (11, 18), but orangutans are currently only found, as distinct species, in Borneo (*P. pygmaeus*) and Sumatra (*Pongo abelii*). The ancestors of orangutans therefore probably migrated from the mainland to Sumatra and from there to Borneo (12), yet it remains unclear when and how these colonization events took place.

It is also unclear how the exceptional environmental features of Sundaland, combined with the characteristic behavioral and ecological traits of orangutans, have shaped their phylogeography. For instance, isolation in refugia or through riverine barriers have been described as important forces underlying the genetic structure of some of the African great apes (20–22), yet the evolutionary history of orangutans remains unresolved. First, the high genetic differentiation between Bornean and Sumatran orangutans (17, 23) is intriguing given recurrent land bridge formation between the islands during the Pleistocene glacial periods (5). Second, within Borneo, arguments for a stable distribution since colonization (24) clash with that of a bottleneck possibly associated

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with the last eruption of Mount Toba (25). Third, the three Bornean subspecies (*P. p. pygmaeus, P. p. wurmbii, P. p. morio*), described on the basis of morphological characteristics (26), show unexplained genetic substructuring (17). Fourth, as for geographical features, the marked role of rivers as dispersal barriers has been highlighted in the study of populations in Sabah (27, 28), but it remains to be seen whether other rivers have had similar vicariant effects. Thus, the relative importance of the Pleistocene sea level and vegetation changes, Toba eruptions, and rivers as dispersal barriers, against the background of regular dispersal behavior of orangutans, remains unknown.

These questions also acquire special relevance today from a conservation perspective, in the light of ongoing habitat conversion (29) and predicted future climate change (30, 31), particularly for insular endemics and highly endangered species such as orangutans.

We recently obtained noninvasively collected wild Bornean orangutan samples from seven long-term study sites, as well as other localities, thus encompassing most of the species' range. Capitalizing on the most extensive sample size to date, we provide genetic evidence for a recent radiation of Bornean populations within the Middle to Late Pleistocene. We further illustrate the role of rivers and sex-biased dispersal in generating the marked population structure of the largest arboreal primate.

Results

mtDNA Analyses. We generated a phylogenetic tree for the mitochondrial (mtDNA) haplotypes from 211 individuals distributed throughout 10 sampling sites in Borneo (Fig. 1*B*), as well as six Sumatran individuals. The tree (Fig. 1*A*) shows a monophyletic Bornean clade with a surprisingly recent mean coalescence date of 176 ka (95% highest posterior density, 72–322 ka), contrasting with a much older estimate from a previous study (17). The phylogenetic tree and divergence estimate further illustrate the deeper coalescence of Bornean and Sumatran haplotypes (mean, 3.6 Ma; 95% highest posterior density, 2.3–5.0 Ma). Given the recurrent formation of potential connections between the islands, these findings point to an unexpectedly recent and single origin for current Bornean populations. Furthermore, the Bornean subspecies, as currently recognized on the basis of morphological

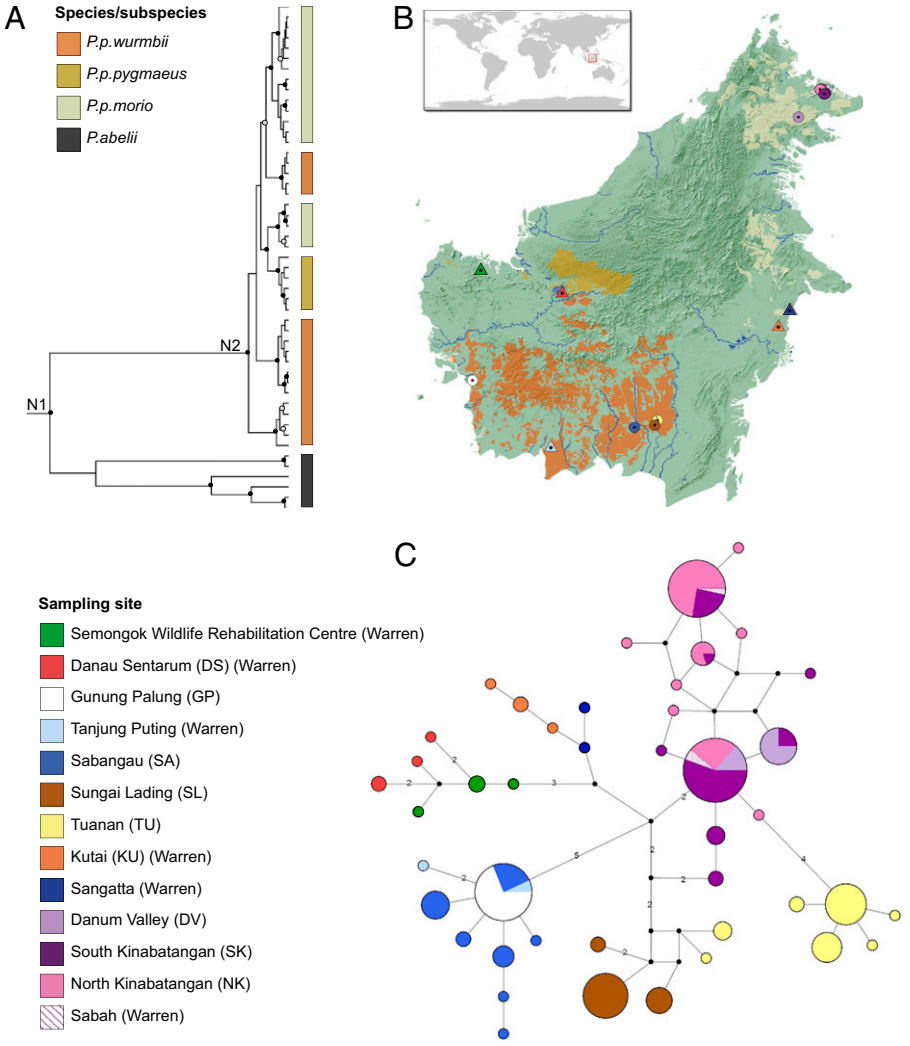


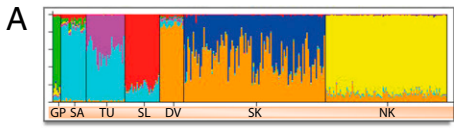
Fig. 1. Phylogenetic reconstruction and sampling sites of Bornean orangutans. (*A*) Bayesian phylogenetic tree of Bornean and Sumatran mtDNA haplotypes. Circles show posterior probabilities (>0.5, open circles; >0.75, black circles). Colored bars next to tips indicate species/subspecies designation. (*B*) Map of Borneo with location of sampling sites. Triangles correspond to sites for which only mtDNA data are available, circles correspond to sites for which additionally microsatellite data are available. Colored ranges on the map represent subspecies. (*C*) Median joining network of Bornean mtDNA HVRI haplotypes. Mutational steps are one unless indicated by the numbers. Two haplotypes from TU more closely related to those from SL are exclusively found in males. Sites with resequenced data from Warren et al. (17) are indicated in parentheses.

characteristics, are not reciprocally monophyletic, and should therefore be reconsidered.

The surprisingly recent radiation of a single Bornean lineage calls for a more detailed exploration of Bornean phylogeography. We generated an mtDNA phylogenetic network (Fig. 1*C*), more appropriate for population level studies than phylogenetic trees as they do not force possible ancestral haplotypes to the tips (32, 33). The network revealed seven main star-like geographical clusters, reflecting considerable structuring within the different subspecies. These seven clusters were further supported by a spatial analysis of molecular variance (SAMOVA), which defines groups of populations that are "geographically homogeneous and maximally differentiated from each other" (34). The analysis indicated that among-group variance asymptotes at 79.27% (F_{CT} = 0.793, $P < 0.01$) with seven groups of populations. The grouping corresponds to an almost complete separation of all sampled sites except for: (*i*) Danum Valley (DV), which clusters with South Kinabatangan (SK), a site in close proximity (approximately 90 km) not separated by geographical barriers (Fig. 1*B*); and (*ii*) Gunung Palung (GP), clustering with Sabangau (SA), a site with which it shares its only haplotype. Our results point to strong interpopulation differentiation for mtDNA, as corroborated by the high and significant Φ_{ST} values for all 36 population pairs (Fig. 2*B*). The exceptions are three lower, albeit still significant, Φ_{ST} values between the sites that share haplotypes. Given the heavy reliance of Φ_{ST} and other classic moment-based estimators on intrapopulation diversity (35), we also computed population average pairwise differences (Table S1). We found generally higher levels of diversity between populations than within, providing additional support for interpopulation differentiation.

Microsatellite Analyses. We also examined differentiation patterns using nuclear loci, which are biparentally inherited and therefore representative of both male and female histories, for the seven sites for which we could generate microsatellite genotypes. Both cluster analyses with Structure and significant pairwise population F_{ST} values indicate strong structuring of these sites (Fig. 2), particularly when separated by rivers (Fig. 1*B*). The structure runs for all seven sites using 12 microsatellite loci (dataset II, Fig. 2*A*) yielded the highest probability runs for K = 7 [Log likelihood (LnL), −9,619.88], partitioning each of the sites as a distinct cluster. Likewise, a more detailed analysis for the five sites for which 25 microsatellite loci were available (dataset I) also led to each one being inferred as a separate cluster (Fig. S1). Generally, high pairwise F_{ST} and level of structuring of populations is congruent with our mtDNA results. However, the cluster analyses using nuclear loci indicate some heterogeneity within populations. As haplotype sharing is rare among populations exchanging migrants, the low levels of gene flow are most likely male-mediated.

We investigated the signature of sex-specific demographic processes more directly by comparing isolation by distance patterns for the nuclear and mtDNA loci. The Mantel test for the relationship between genetic and Euclidean geographical distance yielded a significant and positive correlation for both the nuclear markers and mtDNA (F_{ST}, $r = 0.415$, $P < 0.05$; Φ_{ST}, $r = 0.357$, $P < 0.05$). We also explored the effect of rivers in a partial Mantel test of the association between genetic and cost path distances while controlling for Euclidean distance. Results were significant for the mtDNA ($P < 0.01$; $r = 0.403$) but not the nuclear markers ($P = 0.633$; $r = -0.096$). It is noteworthy, however, that for the mtDNA, only three of the 36 population pairs studied have low Φ_{ST} values (<0.6). Therefore, most populations are highly differentiated from each other despite the short geographical distances between them.



B

	DS	KU	GP	SA	SL	TU	DV	SK	NK
DS		-	-	-	-	-	-	-	-
KU	0.790*		-	-	-	-	-	-	-
GP	0.962	0.986		0.143**	0.160	0.163	0.209**	0.131	0.125
SA	0.880	0.882	0.159		0.110	0.049	0.152	0.083	0.069
SL	0.881	0.874	0.934	0.895		0.084	0.190	0.109	0.121
TU	0.758	0.680	0.827	0.801	0.779		0.117	0.072	0.090
DV	0.910	0.914	0.970	0.893	0.886	0.658		0.036	0.112
SK	0.812	0.754	0.845	0.810	0.814	0.628	0.163**		0.040
NK	0.879	0.854	0.896	0.859	0.879	0.748	0.689	0.409	

Fig. 2. Population structure based on nuclear microsatellite markers. (*A*) Structure run for the seven study sites with 12 microsatellite marker data (dataset II) at K = 7 (LnL, −9,576.8). (*B*) Interpopulation differentiation with pairwise F_{ST} estimates are above the diagonal and pairwise Φ_{ST} estimates are below the diagonal. All are significant at $P < 0.001$ except when indicated (*$P < 0.05$; ** $P < 0.01$).

Discussion

We investigated the evolutionary history of Bornean orangutans using the most comprehensive Bornean sample set compiled to date to our knowledge. Our mtDNA results indicate a surprisingly recent origin for current Bornean populations, and together with the nuclear markers, illustrate that their current distribution has been uniquely shaped by a combination of historical, geographical, and sociobehavioral factors.

Historical Factors: Recent Radiation of Bornean Populations. The recent coalescence of Bornean orangutan haplotypes in the Middle to Late Pleistocene is in striking contrast with that of the other Bornean canopy-bound rainforest species for which data are available, the gibbon *Hylobates muelleri.* This gibbon, distributed throughout the north, east, and west of Kalimantan, has a time to the most recent common ancestor (TMRCA) of 1.78 Ma (95% CI, 1.33–2.25) (36), suggesting that Bornean gibbons have been differentiating within the island for much longer than orangutans. Moreover, Sulawesi macaques (genus *Macaca*), whose ancestors dispersed from Borneo, have a TMRCA with their Bornean sister species of approximately 2 Ma (37). Although the exact timing of their migration is uncertain, the older mtDNA coalescence dates for both Bornean gibbons and Bornean and Sulawesi macaques suggests they have been in Borneo as far back as the Early Pleistocene. Therefore, it is conceivable that orangutans also arrived in Borneo around the same time. Yet, current Bornean orangutan mtDNA haplotypes stem from a very recent common ancestor originating in the Middle to Late Pleistocene.

The relatively short time to the most recent common ancestor of Bornean haplotypes is particularly striking given the deep Bornean–Sumatran orangutan coalescence approximately 3.5 Ma. Such a long differentiation between Bornean and Sumatran haplotypes appears hard to reconcile with the recent episodes of interconnectedness between the islands during the Pleistocene glaciations, most notably during the Last Glacial Maximum approximately 17 ka (2, 5). However, the presence of land bridges does not necessarily imply suitable conditions for migration. A savannah corridor (8) combined with riverine barriers dissecting the exposed land (5, 11) would have presented severe obstacles to migration for orangutans, restricting them to riverine forest galleries along the banks. Coalescence for Bornean and Sumatran haplotypes is expected to vary across species, reflecting differences in dispersal abilities, habitat requirements, or ancestral effective population size, aside from possible discrepancies in dating methods (38). For instance, the south Bornean gibbon *Hylobates albibarbis* and the Sumatran–Malaysian gibbon *Hylobates agilis* have a TMRCA of 1.56 Ma (36), and Bornean and Sumatran pigtailed macaques have one of 3 to 4 Ma (37). By contrast, the Bornean–Sumatran common ancestor of both the silvered langur

(39) and clouded leopard (40) is much more recent than that of orangutans, gibbons, and pig-tailed macaques, probably because of a higher flexibility in habitat use.

Assuming that orangutans arrived in Borneo around the same time as gibbons and macaques, the recent coalescence of Bornean orangutans could be explained by a bottleneck through a severe rainforest contraction. Such a bottleneck would have had a more dramatic impact on the mtDNA structure of orangutans compared with other species as a result of their low densities and slow life histories (18) as well as habitat requirements. Gibbons were apparently not affected by habitat changes as harshly, perhaps because populations can survive in smaller patches. Our findings are consistent with the survival and expansion of a single lineage from within a refugium in Borneo. Geomorphological and palynological data indicate the presence of dryer, more open vegetation in southern and western Borneo during the last glaciation (2, 41), and by extrapolation also during other glaciations (but c.f. refs. 42, 43). Climate change was especially severe during an extended cold period within the penultimate glaciation between 130 and 190 ka (44, 45), which occurred approximately at the time of mean coalescence of Bornean mtDNA haplotypes. More recently, the last Toba eruption approximately 74 ka resulted in a short, albeit significant, decrease in regional temperatures, ensued by a 1,800-y cold stadial (9, 10). Our data do not provide clear signals to make conclusive statements about potential Toba effects. Nonetheless, the coldest period of the penultimate glaciation (44, 45) was more prolonged than the cold period following the last Toba eruption, suggesting more severe effects of the former on the extent of rainforest across Sundaland. In any event, suitable rainforest habitat for orangutans should have existed in certain regions in Borneo where a refugium population survived the dry glacial conditions. Possible Pleistocene refugia in Borneo have also been described for numerous other rainforest species such as termites, ants, orchids, oaks, and large-bodied mammals (37, 46–51), and together with the isolation of the island, could act as a mechanism of evolutionary diversification driving high Bornean species endemism. Following the expansion of orangutans throughout the island, the Pleistocene climatic oscillations should have led to recurrent population expansions and contractions.

Geographical and Sociobehavioral Barriers. Despite the recent common ancestry of Bornean populations, our analyses revealed high and significant mitochondrial differentiation, with populations within currently recognized subspecies generally displaying as much differentiation as those between subspecies. Of notable interest is the great extent of subdivision and lack of reciprocal monophyly for the morphologically recognized subspecies *P. p. morio* and *P. p. wurmbii*. MtDNA haplotype sharing is uncommon and for populations separated by rivers occurs only in two instances: (i) for SA and GP and (ii) for the northern and southern populations across the Kinabatangan river. In both cases, very recent common ancestry could explain the incomplete mtDNA lineage sorting. For North Kinabatangan (NK) and SK, Jalil et al. (27) proposed an expansion from a recent common refugium further west in Mount Kinabalu, as posited for other Bornean species (46, 47, 49). DV, with its low haplotype diversity, might also be the result of a recent range expansion. GP is located proximally to the Bangka–Belitung–Karimata–Schwaner divide, from where orangutans are presumed to have dispersed to the rest of Borneo (12) and where we might expect a rich haplotype diversity. However, the presence of only one mtDNA haplotype shared with populations further east suggests that the current population in GP is recent and/or underwent a severe recent bottleneck. This and other local bottlenecks make it impossible to reconstruct a colonization of Borneo through the southwestern "choke point" (52).

The rarity of mtDNA haplotype sharing among Bornean populations contrasts with patterns in the patrilocal chimpanzees and bonobos (53, 54), where mtDNA sharing is extensive. Interestingly, two orangutan haplotypes from one site (Tuanan, TU) that were more closely related to those of another site (Sungai Lading, SL) pertain only to males. Although nuclear differentiation among the orangutan populations is significant, we find evidence for a small degree of nuclear gene flow, suggesting that it is male-mediated. Furthermore, the effect of rivers on the isolation by distance patterns for the mtDNA indicate that these are important barriers to female movement, probably as a result of smaller dispersal distances of females (18). An association between mtDNA genetic distance and distances around rivers has also been found in gorillas (20), and a role for differential dispersal distances between the sexes has been posited for western lowland gorillas (55). Our results are consistent with the pattern of female philopatry and male-biased dispersal proposed by Delgado and van Schaik (18) and indicate that the orangutan sexes are subject to very different constraints on mobility. Although female philopatric behavior may be responsible for the strong effect of geographical barriers on mtDNA structure, we cannot make any conclusive statements on the effects of rivers on males. More continuous sampling, especially along rivers and examination of Y-chromosomal markers, representative of male histories, will prove useful in determining how geographical barriers differentially affect the sexes. In addition, further geomorphological data on river course and width changes through time would contribute to the understanding of their vicariant action.

Bornean orangutan distribution and population structure has been uniquely shaped by the Pleistocene fluctuations and by sociobehavioral and geographical barriers to movement. Our findings support a recent radiation of Bornean orangutans in the Middle to Late Pleistocene, resulting in "static" clusters of females strongly separated by geographical barriers and subject to high differentiation, with more mobile males exerting a homogenizing influence on the nuclear gene pool. Further sampling will help establish whether there is a marker specific pattern of clusters versus clines resulting from sex-biased dispersal (c.f ref. 52). In addition, in depth population genetic studies of other endangered and endemic taxa such as the Bornean gibbons and Sumatran orangutans will be of interest in contrasting the differential effects of environmental processes.

Materials and Methods

Samples and Datasets. Our data comprise noninvasively collected fecal and hair samples from a number of long-term study sites: Gunung Palung (GP), Sabangau (SA), Sungai Lading (SL), Tuanan (TU), Danum Valley Conservation Area (DV), and the Lower Kinabatangan Wildlife Sanctuary (Fig. 1*B*). We partitioned the latter site into South Kinabatangan (SK) and North Kinabatangan (NK), given the significant differentiation between the locales found by Goossens et al. (28). In addition, we incorporated scattered samples from Warren et al. (17) (Table S2), encompassing most of the current distribution of *P. pygmaeus* (Fig. 1*B*). Depending on sample quality and data availability, we used two different datasets for mtDNA analyses, and two for nuclear microsatellite analyses (Table S3). DNA extraction and quantification procedures are described in *SI Materials and Methods*.

mtDNA Analyses. Based on unique microsatellite genotypes or mtDNA haplotypes (*SI Materials and Methods*), we obtained the following long-term study site sample sizes: SA (n = 23), SL (n = 26), TU (n = 30), and DV (n = 18). We also sequenced low DNA quantity samples from GP (n = 20), where individual identification was done through long-term observational data. Additionally, haplotypes for individuals from SK (n = 38) and NK (n = 35) were from Jalil et al. (27) (GenBank accession numbers EU547189–EU547201). Finally, we resequenced 21 extracts from the Bornean samples in Warren et al. (17) to cover the same region of mtDNA (Table S2). We sequenced a 323-bp region of the mtDNA hypervariable region I (HVRI). Details on the primers and PCR conditions and raw data analyses are provided in *SI Materials and Methods*. Summary statistics including haplotype diversity (h_d), nucleotide diversity (π), and average pairwise differences were calculated in DNAsp 5 (56) and Arlequin 3.11 (57). We conducted model selection tests on jModelTest 0.1 (58, 59), using the Akaike information criterion to choose the most suitable model and its parameters.

For the phylogenetic analyses, we incorporated HVRI haplotypes from all long-term study sites as well as Warren resequenced samples (Tables S2 and S3). First, to infer the coalescence date for Bornean mtDNA haplotypes, we used a Bayesian Markov chain Monte Carlo analysis as implemented in BEAST 1.5.4 (60) and produced a phylogenetic tree. We included the collapsed haplotypes from 211 Bornean and six Sumatran orangutans, as well as 19 humans as an outgroup. Based on the Akaike information criterion from jModeltest, we selected the HKY + G model. We used an uncorrelated relaxed log-normal clock (61), specifying a normal distribution with a mean HVRI substitution rate of 0.1643 substitutions per nucleotide per Myr for the mean rate prior. We chose this corrected HVRI estimate (62) because it takes into account the effects of purifying selection on the entire mtDNA molecule as well as saturation factors affecting the molecular rate decay described in numerous studies (38, 63, 64), and is therefore appropriate for population-level analyses (62, 65). The 95% confidence interval for the normal distribution spanned HVRI substitution rates obtained in other studies, from 0.06 to 0.25 substitutions/site/Myr (66). Using the birth-death prior for branching rates, we carried out two runs for 25 million generations with parameter sampling every 1,000 generations. Tracer 1.4.1 (67) was then used to examine whether the 10% burn-in period and effective sample sizes were adequate. Both runs were combined in LogCombiner 1.4.8, and the resulting tree visualized and edited using Figtree 1.2 (68), omitting human haplotypes. Second, to infer the coalescence date for Bornean and Sumatran mtDNA haplotypes, we used the same procedure, but instead of the corrected mutation rate, we chose two fossil based divergence estimates as priors. Fossil calibration points provide estimates of phylogenetic rates suitable for analyses at the interspecific level (65). The two calibration points were the Ponginae-Homininae divergence at approximately 14 Ma (69, 70) and the *Pan-Homo* divergence older than 6 Ma (71, 72). We specified log-normally distributed priors, appropriate for paleontological data (73). For the Ponginae-Homininae divergence, we used a log-normal mean of 0, log-normal SD of 0.56, and offset of 13 Ma, thereby obtaining a broad distribution with a 95% interval from 13.4 to 20 Ma. This range incorporates the uncertainties associated with the upper bound estimate of a split. For the *Pan-Homo* calibration, we used a log-normal mean of 0, log-normal SD of 0.56, and offset of 5 Ma, spanning a 95% interval from 5.4 to 7.5 Ma. The tree topology remained the same as in the first analysis, so it is not presented. Third, we investigated phylogenetic relationships at the intraspecific level by generating a median-joining network for the Bornean haplotypes using Network 4.0 (74).

For the population structure analyses, we used data from the long-term study sites GP, SA, SL, TU, DV, NK, and SK. In addition, we incorporated Danau Sentarum (DS) and Kutai (KU) sampling sites from Warren et al. (17) for which at least three samples of precise origin are available (cf. ref. 20; Table S2). We calculated pairwise Φ_{ST} values in Arlequin, using the Tamura Nei model (75) and a γ distribution shape parameter of 0.344. We obtained significance levels using 10,000 permutations. To define the most differentiated groups of populations, we also performed a spatial analysis of molecular variance (SAMOVA) with SAMOVA software, version 1.0 (34), using previously published geographical coordinates (17, 76).

Microsatellite Analyses. Microsatellite analyses focused only on samples from long-term study sites GP, SA, SL, TU, DV, SK, and NK. For the low DNA quality and quantity samples from GP, we could obtain genotypes for six individuals. We genotyped samples from all sites except SK and NK using a panel of 25 highly polymorphic nuclear microsatellite markers (28, 77) listed in Table S4, following the protocol given in *SI Materials and Methods*. Additionally, we incorporated previously generated data from NK and SK for 12 microsatellite markers (28), which were part of our panel of 25 markers. We standardized the data and performed identity analyses as described in *SI Materials and Methods*. After this procedure, we obtained two data sets: (*i*) dataset I includes 25 markers and 98 individuals from the five study sites GP (n = 6), SA (n = 19), SL (n = 26), TU (n = 29), and DV (n = 18); and (*ii*) dataset II includes 12 markers and 295 individuals from seven study sites, including all from dataset I plus NK (n = 91) and SK (n = 106).

After Bonferroni correction, we found no deviation from Hardy–Weinberg equilibrium, and only four pairs of different loci from two populations showed linkage disequilibrium, which is most likely explained by demographic effects rather than linkage. Also, we found evidence for possible null alleles for one locus in one population. As it was not consistent across populations, we did not exclude this locus from further analyses.

We used Genetix 4.05 (78) to obtain population pairwise F_{ST} values and significance levels. We also performed two separate analyses on Structure 2.3 (79) using the admixture model with correlated allele frequencies, and the Locprior model, which improves clustering when the signal is weak without spuriously inferring structure if absent (80). We specified a burn-in length of 10^5 followed by 10^6 Markov chain Monte Carlo steps. For each K, we ran the analysis 10 times. In the first analysis, we incorporated the widely distributed seven populations genotyped at 12 microsatellite markers (dataset II). In the second analysis, we further refined our findings focusing on the five populations for which we have genotypes for 25 microsatellite markers (dataset I).

We calculated geographical distance matrices as Euclidean and cost path distances between all study populations. The latter, representing true surface distances circumnavigating riverine barriers, were computed from the Shuttle Radar Topography Mission global Digital Elevation Model, as distributed by ESRI (81). We clipped the Digital Elevation Model to encompass the whole of Borneo and filled sinks to obtain a depressionless elevation model, which was then reprojected into the Universal Transverse Mercator coordinate system with a resolution of 100 m. From this, we constructed a flow accumulation raster and extracted grid cells with values of at least 1,000 to generate a stream order raster following the convention of Strahler (82). We then produced a cost raster by designating areas with flow accumulation values lower than 1,000 and streams of order 1 to 2, a cost of 1, whereas streams of orders 3, 4, and 5 were assigned costs of 3,000, 4,000, and 5,000, respectively. Streams of order 6 to 7 were designated as uncrossable barriers (cf. ref. 20). After masking the resulting cost raster with the Shuttle Radar Topography Mission Water dataset (81), we calculated dyadic cost path distances between the study populations. For all geospatial analyses, we used ArcInfo Spatial Analyst extension for ArcGIS 9.3 (83).

To investigate the association between genetic (pairwise Φ_{ST} for HVRI and F_{ST} for microsatellite markers) and geographical distances (Euclidean and cost path), we performed (partial) Mantel tests in R 2.10.1 (84), using the "ecodist" package (85).

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Supporting Information

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SI Materials and Methods

DNA Extraction and Quantification. We extracted DNA using the QIAamp DNA Stool Mini Kit (Qiagen) following the manufacturer's protocol with one modification: samples were allowed to incubate for a minimum of 30 min before elution. We quantified DNA through real-time quantitative PCR using the protocol from a previous study (1). The real-time PCR assay allows determination of the number of positive PCR replicates per extract necessary to obtain a 99% confidence level that a homozygous genotype is correct (1). For a heterozygous genotype, our criterion was the observation of each of the two alleles at least twice in independent PCRs.

mtDNA Analyses. We sequenced a 323-bp region of the mtDNA HVRI using the primers DLF (5′-CCT GCC CCT GTA GTA CAA ATA AGT A-3′) and D5 (2). PCR amplifications were performed in a 20-μL reaction volume containing 0.25 μM of each primer, 0.2 mM dNTPs, 1× PCR buffer (Qiagen), 2 μL BSA, 0.5 U HotStarTaq DNA polymerase (Qiagen), and 1 μL template DNA. PCR conditions were as follows: initial denaturation at 95 °C for 15 min, followed by 45 cycles of 94 °C for 40 s, 52 °C for 30 s, 72 °C for 30 s, and final extension at 72 °C for 10 min. Reactions were purified with the QIAquick PCR Purification Kit (Qiagen) following the manufacturer's recommendations. Cycle sequencing was performed in a 10 μL reaction volume containing 1 μL of purified PCR product, 1× sequencing buffer (80 mM Tris, 2 mM $MgCl_2$, pH 9.0), 0.4 μM forward primer, and 0.3 μL BigDye Terminator, version 3.1. The cycle sequencing conditions were initial denaturation at 95 °C for 45 seconds, followed by 30 cycles of 95 °C for 30 s, 52 °C for 20 s, and 60 °C for 2 min. Capillary electrophoresis was carried out using the 3730 DNA analyzer (Applied Biosystems).

All raw data were viewed and edited in Sequencing Analysis 5.2 (Applied Biosystems). The sequences were aligned with ClustalW (3) in Bioedit 7.0.9.0 (4) and collapsed using DAMBE 5.0.7.2 (5). Unique DNA sequences have been submitted to the EMBL database under accession numbers FR717918–FR717940. The resequenced samples from Warren et al. (2) have been updated maintaining former accession numbers.

Microsatellite Analyses. To standardize the data sets from NK and SK, we genotyped at least 10 original extracts from NK and SK for each of the 12 loci. We ran these samples on the same instruments and analyzed them in the same way as for the other study sites. In cases in which NK and SK genotypes were found to differ as a result of bin set discrepancies, we adjusted allele sizes to match our current bin set. We were not able to complete genotypes for all 25 markers as a result of low DNA quantity and quality, probably because of long-term storage degradation.

All samples from all sites were subjected to identity analyses on Cervus 3.0 (6, 7). If identical genotypes were found, only one was included in our data set.

PCR amplifications were performed as multiplex reactions in an 8 μL volume containing 1 μL DNA, 4 μL Multiplex Master Mix (Qiagen), 0.8 μL primer mix, and 2.2 μL water. Amplification conditions were: initial denaturation at 95 °C for 15 min, followed by 40 cycles of 94 °C for 30 s, 58 °C for 90 s, 72 °C for 1 min, and a final extension at 60 °C for 30 min.

We performed capillary electrophoresis on the 3730xl DNA Analyzer (Applied Biosystems). Products were analyzed using GeneMapper version 4.0 (Applied Biosystems).

We used Arlequin 3.11 to calculate deviation from Hardy–Weinberg equilibrium and GenePop 4.0 (8, 9) to assess linkage disequilibrium. We checked for allelic dropout and null alleles using ML-NullFrEq (10).

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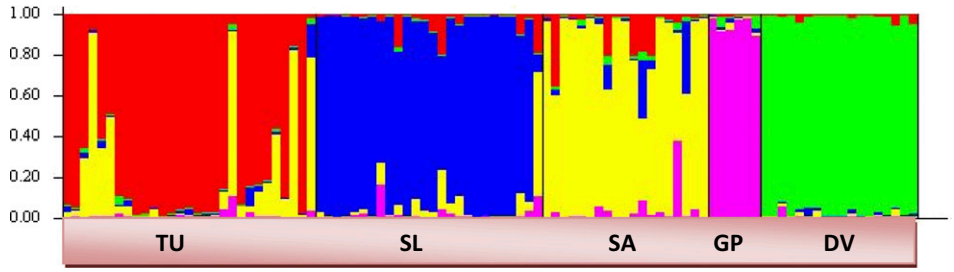

Fig. S1. Structure run for the five study sites with 25 microsatellite marker data (dataset I) at K = 5 (LnL, −5,395.4).

Table S1. Population average pairwise differences calculated with Arlequin 3.11

Site	GP	SA	SL	TU	DS	KU	DV	SK	NK
GP	0*	0.86	13.41	12.24	11.81	10.54	8.72	8.95	9.89
SA	0.15	1.42*	14.27	13.23	12.11	11.54	9.64	9.91	10.99
SL	12.63	12.78	1.56*	11.96	12.99	11.82	10.01	10.33	13.35
TU	10.44	10.72	9.38	3.6*	11.28	9.73	6.85	7.61	10.14
DS	13.57	14.57	15.53	14.84	3.51*	10.87	11.7	12.58	15.34
KU	10.02	10.31	10.52	7.42	8.6	1.04*	7.34	8.17	10.72
DV	8.44	8.66	8.96	4.78	9.67	6.55	0.54*	1.71	3.93
SK	7.86	8.11	8.46	4.72	9.73	6.56	0.35	2.18*	3.23
NK	9.07	9.47	11.75	7.52	12.77	9.39	2.84	1.33	1.64*

*Diagonal elements represent average number of intrapopulation pairwise differences. Above this diagonal line are average number of interpopulation pairwise differences, and below are corrected average interpopulation pairwise differences, computed using Tamura-Nei γ distribution with shape parameter 0.344.

Table S2. Samples from the Warren et al. (2) dataset included in analyses after resequencing (unchanged EMBL accession numbers)

Code	Status	Origin/reference	Site assigned
OU TNK41	W	Kutai National Park, EK	KU
OU TNK39	W	Kutai National Park, EK	KU
OU TNK37	W	Kutai National Park, EK	KU
OU TNK36	W	Kutai National Park, EK	KU
OU TP14	R	Tanjung Puting, CK	None
OU TP6	W	Tanjung Puting, CK	None
OU TP24	W	Tanjung Puting, CK	None
OU DSRA	W	Danau Sentarum, NK	DS
OU DSLE1	W	Danau Sentarum, NK	DS
OU DSME1	W	Danau Sentarum, NK	DS
OU DSME2	W	Danau Sentarum, NK	DS
OU SEUA	R	Semongok, Sarawak	None
OU SEBU	R	Semongok, Sarawak	None
OU SEOA	R	Semongok, Sarawak	None
OU SE8	R	Semongok, Sarawak	None
OU KPC	W	Sangatta, EK	None
OU KAI	W	Sangatta, EK	None
OU SB71	W	Sandakan, Sabah	None
OU SB60	W	Kinabatangan, Sabah	None
OU SB57	W	Sukau, Kinabatangan, Sabah	None
OU SB372	R	Sepilok, Sabah	None

Table modified from Warren et al. (2). CK, central Kalimantan; EK, east Kalimantan; NK, north Kalimantan; KU, Kutai; DS, Danau Sentarum; R, rehabilitation center; W, wild.

Table S3. mtDNA and nuclear microsatellite datasets

Marker	Analyses	Samples included
mtDNA	Phylogenetic reconstruction	Resequenced Warren dataset and GP, SA, SL, TU, DV, SK, and NK
mtDNA	Population structure	Resequenced Warren sites KU and DS, and GP, SA, SL, TU, DV, SK, and NK
Nuclear microsatellites	Population structure	GP, SA, SL, TU, and DV (25 microsatellite loci)
Nuclear microsatellites	Population structure	GP, SA, SL, TU, DV, SK, and NK (12 microsatellite loci)

Table S4. Nuclear microsatellite loci amplified in the study

Locus name	Sequence (5′-3′)	Repeat	Reference
D1S550	F: CCTGTTGCCACCTACAAAAG	Tetranucleotide	1
D1S550	R: TAAGTTAGTTCAAATTCATCAGTGC	Tetranucleotide	1
D2S1326	F: AGACAGTCAAGAATAACTGCCC	Tetranucleotide	1
D2S1326	R: CTGTGGCTCAAAAGCTGAAT	Tetranucleotide	1
D3S2459	F: CTGGTTTGGGTCTGTTATGG	Tetranucleotide	1
D3S2459	R: AGGGACTTAGAAAGATAGCAGG	Tetranucleotide	1
D4S1627	F: AGCATTAGCATTTGTCCTGG	Tetranucleotide	1
D4S1627	R: GACTAACCTGACTCCCCCTC	Tetranucleotide	1
D4S2408	F: AATAAACTTCAACTTCAATTCATCC	Tetranucleotide	1
D4S2408	R: AGGTAAAGGCTCTTCTTGGC	Tetranucleotide	1
D5S1470	F: CATGCACAGTGTGTTTACTGG	Tetranucleotide	1
D5S1470	R: TAGGATTTTACTATATTCCCCAGG	Tetranucleotide	1
D13S321	F: TACCAACATGTTCATTGTAGATAGA	Tetranucleotide	1
D13S321	R: CATACACCTGTGGACCCATC	Tetranucleotide	1
D13S765	F: TGTAACTTACTTCAAATGGCTCA	Tetranucleotide	1
D13S765	R: TTGAAACTTACAGACAGCTTGC	Tetranucleotide	1
D16S420	F: ATTTCCTGAGGTCTAAAGCACCC	Dinucleotide	1
D16S420	R: TTAGGCCCAGTCCACACTCAAG	Dinucleotide	1
D2S141	F: ACTAATTACTACCCNCACTCCC	Dinucleotide	1
D2S141	R: TTTTCCAAACAGATACAGTGAACTT	Dinucleotide	1
D5S1505	F: TAAGTGCCAGAGTCTCCCAC	Tetranucleotide	1
D5S1505	R: TAAGGCATGTCTCGGAGCTA	Tetranucleotide	1
D6S501	F: GCTGGAAACTGATAAGGGCT	Tetranucleotide	1
D6S501	R: GCCACCCTGGCTAAGTTACT	Tetranucleotide	1
D5S1457	F: TAGGTTCTGGGCATGTCTGT	Tetranucleotide	1
D5S1457	R: TGCTTGGCACACTTCAGG	Tetranucleotide	1
O4_6	F: GGCAATGTAACATATCCCTCTGTGT	Tetranucleotide	2
O4_6	R: AGCCATGGACCTTGTGAGAAAAG	Tetranucleotide	2
O4_A5	F: ATGGGCCCAGAAAACAACTCAGT	Tetranucleotide	2
O4_A5	R: AGATAAAGGAATGGATAGATGGACAGA	Tetranucleotide	2
O4_B5	F: GAGCCCTGATTCGTTTTACTGG	Tetranucleotide	2
O4_B5	R: AGCAAAGGCAGAAAACTGTAATGA	Tetranucleotide	2
O4_B6	F: TGGAGCCTGAATATGTGACTGAAT	Tetranucleotide	2
O4_B6	R: AATGCCAGGATTTCCTTCTTTTT	Tetranucleotide	2
O4_A7	F: ACTGGCCCATTCAAAGTCTGTCATT	Tetranucleotide	2
O4_A7	R: ACTGGCCCATTCAAAGTCTGT	Tetranucleotide	2
O4_A1	F: CTCCCCTTCCTTCCTTTATTCAGTT	Tetranucleotide	2
O4_A1	R: CAACACTTGGCAGTCACAAATCAG	Tetranucleotide	2
O4_B17	F: GTACCGACGGTGCACGAACAATGTA	Tetranucleotide	2
O4_B17	R: AGCCTGGCTGAAAAGTGGAACTGAG	Tetranucleotide	2
O4_B20	F: CCTGCATTTTGTCACTCCCTCAACC	Tetranucleotide	2
O4_B20	R: CTGCCACACCTCCATGGACACAGAT	Tetranucleotide	2
O4_C13	F: CTGGGCACACTGTATATGGGGTAG	Tetranucleotide	2
O4_C13	R: GTTTGAGACCACTCATGATGCAAAGACC	Tetranucleotide	2
O4_C9	F: TGCAGGCCAGGGCTTCTTTCAA	Tetranucleotide	2
O4_C9	R: CAGTCTCCCCAGGACCCCTACACAG	Tetranucleotide	2
O4_Chr5	F: CAGCAGCTCCTGAAATATCTGTCC	Tetranucleotide	2
O4_Chr5	R: GTTTGGGGTAGAGGAAAGCAGGTTGAT	Tetranucleotide	2
O4_Chr7	F: CATCTCTTTATGGCTGACTGTTGAT	Tetranucleotide	2
O4_Chr7	R: GTTTGGTCCAAGACAAATTTGTATGAGT	Tetranucleotide	2

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Parentage-based pedigree reconstruction reveals female matrilineal clusters and male-biased dispersal in nongregarious Asian great apes, the Bornean orang-utans (*Pongo pygmaeus*)

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Abstract

Philopatry and sex-biased dispersal have a strong influence on population genetic structure, so the study of species dispersal patterns and evolutionary mechanisms shaping them are of great interest. Particularly nongregarious mammalian species present an underexplored field of study: despite their lower levels of sociality compared to group-living species, interactions among individuals do occur, providing opportunities for cryptic kin selection. Among the least gregarious primates are orang-utans (genus: *Pongo*), in which preferential associations among females have nevertheless been observed, but for which the presence of kin structures was so far unresolved because of the equivocal results of previous genetic studies. To clarify relatedness and dispersal patterns in orang-utans, we examined the largest longitudinal set of individuals with combined genetic, spatial and behavioural data. We found that males had significantly higher mitochondrial DNA (mtDNA) variation and more unique haplotypes, thus underscoring their different maternal ancestries compared to females. Moreover, pedigree reconstruction based on 24 highly polymorphic microsatellite markers and mtDNA haplotypes demonstrated the presence of three matrilineal clusters of generally highly related females with substantially overlapping ranges. In orang-utans and possibly other nongregarious species, comparing average biparental relatedness (*r*) of males and females to infer sex-biased dispersal is extremely problematic. This is because the opportunistic sampling regime frequently employed in nongregarious species, combined with overlapping space use of distinct matrilineal clusters, leads to a strong downward bias when mtDNA lineage membership is ignored. Thus, in nongregarious species, correct inferences of dispersal can only be achieved by combining several genetic approaches with detailed spatial information.

Keywords: kin structure, matrilineal cluster, nongroup-living species, relatedness

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Introduction

Sex-biased natal dispersal, whereby one sex displays a greater tendency to leave or travel longer distances away from the natal area before breeding, is ubiquitous in the animal kingdom (Howard 1960; Clobert *et al.* 2001). This crucial life history trait has a strong impact on population genetic structure, influencing the maintenance and loss of genetic diversity in populations (Chesser 1991b; Sugg *et al.* 1996; Storz 1999). Hence,

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resolving a species' dispersal pattern as well as the mechanisms that drive these is of great interest.

Some of the evolutionary mechanisms invoked to explain the tendency for one sex to exhibit site fidelity or philopatry, i.e. the tendency to breed within or in close proximity to the natal range, include ecological benefits. For instance, philopatric individuals might benefit from familiarity with resources and avoid the risks associated with migration through unknown areas (Greenwood 1980; Lawson Handley & Perrin 2007). Philopatry results in kin structures that might also confer social benefits because of nepotistic interactions, providing inclusive fitness benefits that could augment or even drive philopatry (Perrin & Goudet 2001; Lawson Handley & Perrin 2007). The prediction for species with the mate-defence mating systems prevalent among mammals is that females, who benefit most from acquaintance with a given territory, should be philopatric, with males dispersing to avoid kin competition and inbreeding (Greenwood 1980; Dobson 1982; Pusey 1987; Wolff 1993).

The social organization of group-living mammals has drawn particularly intense interest. In these species, the salient social interactions have prompted many genetic studies to investigate whether kin structures among same-sex members underlie social behaviours such as tolerance, cooperation, learning and cultural variation (spotted hyenas; Van Horn *et al.* 2004; chimpanzees, Lukas *et al.* 2005; horses; Cameron *et al.* 2009; chacma baboons, King *et al.* 2011). Far fewer studies, however, have examined relatedness patterns in nongregarious species. Nevertheless, individuals of nongregarious species may have 'social networks' (Charles-Dominique 1978), engaging in associations with neighbours, so opportunities for cryptic kin selection to operate exist (Hatchwell 2010). Consequently, the exploration of kin structures in such species may lead to important new insights.

The few genetic investigations to date of nongregarious mammals have concentrated on carnivores (raccoons, Ratnayeke *et al.* 2002; cougars, Biek *et al.* 2006; bears, Zedrosser *et al.* 2007) and rodents (woodrats, McEachern *et al.* 2007), as well as a few lemur species among the primates (Kappeler *et al.* 2002; Eberle & Kappeler 2006; Radespiel *et al.* 2009). Such studies have proven invaluable, as illustrated by the examination of the solitarily foraging grey mouse lemur, a species in which females allo-nurse in diurnal sleeping groups. The usage of genetic markers enabled Eberle & Kappeler (2006) to establish that allo-nursing females comprised close maternal relatives, thus providing strong evidence for kin-based communal breeding. In other species without such opportunities for association, nepotistic behaviour could nonetheless still occur albeit in less obvious ways, for instance through reduced aggression and increased tolerance towards relatives that might make settlement in familiar areas easier (Perrin & Goudet 2001; Hatchwell 2010).

Among the most enigmatic nongregarious species are the Asian great apes, the orang-utans (genus: *Pongo*). Like most other great apes, orang-utans have a fission–fusion social system. But they stand out as a result of their especially low levels of sociality (van Schaik 1999) and possibly different social organization. In orang-utans, behavioural evidence points to female philopatry and male-biased dispersal (Galdikas 1985b; Mitani 1989; van Schaik & van Hooff 1996; Delgado & Van Schaik 2000), while in African great apes and humans, female dispersal is common (Eriksson *et al.* 2006; Wilkins & Marlowe 2006; Douadi *et al.* 2007; Langergraber *et al.* 2007; Guschanski *et al.* 2008). Such a dispersal pattern might affect associations among individuals, which despite occurring infrequently, do take place (van Schaik 1999; Delgado & Van Schaik 2000).

Yet the pattern of sex-biased dispersal in orang-utan populations is not clear. Broad-scale studies show tighter geographical clustering of mtDNA compared to Y-chromosome haplotypes across the highly differentiated orang-utan populations (Arora *et al.* 2010; Nietlisbach *et al.* accepted), suggesting historical male-mediated gene flow. Nevertheless, three previously published local scale studies of contemporary dispersal examining relatedness within populations did not confirm this pattern. These studies were based on conventional genetic methodology relying on the comparison of average pairwise relatedness (r) estimates of adult females and adult males obtained using biparentally inherited microsatellite markers. The expectation is that the more philopatric sex comprising related individuals should have higher r values than the dispersing sex comprising immigrants (Prugnolle & de Meeus 2002; Lawson Handley & Perrin 2007). The relatedness comparisons of the three studies were indicative of dispersal of both sexes (Utami *et al.* 2002), philopatry of both sexes (Goossens *et al.* 2006) or male-biased dispersal (Morrogh-Bernard *et al.* 2011). Nevertheless, the inclusion of rehabilitants in the first study, habitat fragmentation in the second study and the smaller sample size in the third study might have been responsible for these differences. The discrepant behavioural and genetic results render the social organization of orang-utans unresolved. It is also unclear whether contemporary dispersal patterns are at odds with historical patterns. Determining whether orang-utans have kin structures and how these are linked to dispersal is crucial step before investigating the possible evolutionary mechanisms underlying the movement of individuals and genes, population genetic structure, and social behaviour.

The aim of the present study was to gain an insight into the dispersal and relatedness patterns of orang-utans, based on the ongoing long-term study at Tuanan Orang-utan Research Area, Borneo, Indonesia. We capitalized on the largest set of genetically characterized sexually mature individuals ($n = 40$) from a natural population of orang-utans to test genetic predictions based on field observations of female philopatry and male-biased dispersal. We included only sexually mature individuals because they have potentially already settled within the natal area or dispersed to breed (Prugnolle & de Meeus 2002; Lawson Handley & Perrin 2007). By complementing spatial and behavioural information, as well as genetic data from the maternally inherited mitochondrial DNA (mtDNA) and a panel of 24 autosomal microsatellite markers, we tested the following predictions:

1. *MtDNA diversity patterns.* Diversity levels are expected to be higher for males if they are the dispersing sex, reflecting their more varied maternal ancestries.
2. *Pedigree relationships.* The number of closely related dyads, and especially maternally related dyads, as estimated from a parentage-based pedigree reconstruction, is expected to be higher among females compared to males.
3. *Average pairwise relatedness estimates.* The estimates are expected to be higher among females than males, as the latter should comprise immigrants.

In addition to disentangling the dispersal patterns in orang-utans, we discuss the effects of sampling regime, life history traits and spatial distribution of individuals on relatedness estimation, which is especially significant when studying nongroup-living animals.

Materials and methods

Study population

Sampling was conducted in the Tuanan Orang-utan Research Area (2°09′ South; 114°26′ East), Mawas Conservation Area, Central Kalimantan, Indonesia. This site is located within a peat swamp forest of approximately 750 ha, accessible through grid-based trails. The orang-utan density estimate for the area is 4.25–4.5 individuals per km^2 (van Schaik *et al.* 2005). Females at this site have home ranges estimated to be 325 ha (±125 ha) (Wartmann *et al.* 2010; van Noordwijk *et al.* 2012). Among males, two morphs are found: flanged males, which have fully developed irreversible secondary sexual characteristics, and unflanged males, which have not (Delgado & Van Schaik 2000; Utami *et al.* 2002). Home ranges of both flanged and unflanged males are far larger than those of females, also exceeding the size of the study site; their sizes are, therefore, unknown (Utami Atmoko *et al.* 2009; van Noordwijk *et al.* 2012).

Behavioural, spatial and genetic data collection

At this longitudinal study site, an intensive sampling regime from 2003 to 2009 targeted the collection of combined behavioural, spatial and genetic data for each individual, following the standard orang-utan protocol (http://www.aim.uzh.ch/orangutannetwork/FieldGuidelines.html). Trained observers conducted over 25 000 h of focal follows, normally nest-to-nest, to record behavioural and spatial information including space use, frequency of sightings, sex and age (Wich *et al.* 2004; van Noordwijk *et al.* 2012). The age of individuals born after 2003 was either known or estimated to the closest year; for individuals born before 2003, age was estimated based on known landmark ages in orang-utans (Wich *et al.* 2004).

Faecal samples were obtained during focal follows of individuals. Multiple samples were collected per individual throughout the study period and throughout the entire study area. We extracted DNA from the faecal samples with the QIAamp DNA Stool Mini Kit (Qiagen) and followed the manufacturer's protocol with a slight modification: elution was preceded by a 30-min incubation period. We genotyped individuals at up to 24 autosomal microsatellite markers and sequenced 450 bp of the hypervariable region I (HVRI) of the mtDNA using the same procedures as described in Arora *et al.* (2010).

For the genotyping, we minimized the genotyping errors associated with low quantity and quality of DNA obtained from noninvasively collected samples through the approach established by Morin *et al.* (2001). This method involves DNA quantification in each extract through real-time quantitative polymerase chain reaction (rtPCR), so as to determine the number of positive PCR replicates required to achieve a 99% certainty in a homozygous genotype. For a heterozygous genotype, the observation of each of the two alleles at least twice in independent PCRs is required. We initially used a panel of six autosomal microsatellite markers to genotype all samples obtained from potentially distinct individuals (Table S1, Supporting information). These markers were chosen because of their low-cumulative nonexclusion probabilities: 1.36×10^{-5} for unrelated individuals and 8.90×10^{-3} for full siblings, as determined by Cervus 3.0 (Kalinowski *et al.* 2007). Usage of these markers allowed us to distinguish unique individuals, providing a genetic method to link the behaviour of followed individuals to their genetic identity in a

longitudinal study. When repeated genotypes were obtained, we discarded all but one to have a data set of distinct individuals. These unique individuals were further genotyped at an additional 18 loci, resulting in a total of 24 autosomal microsatellite markers (Table S1), which were all in Hardy–Weinberg equilibrium, and showed no evidence of linkage disequilibrium or null alleles, as tested using Arlequin 3.11 (Excoffier *et al.* 2005), GenePop 4.0 (Rousset 2008) and ML-NullFreq (Kalinowski & Taper 2006), respectively. Details on the primers and PCR amplification conditions are described in the supporting information. For seven adult males, low autosomal DNA quality and quantity allowed only partial genotypes, restricted to the six markers used in the identity analyses. In total, multi-locus autosomal genotypes were obtained for 19 females and 29 males.

To obtain haplotype information, we sequenced 450 bp of the hypervariable region I (HVRI) of the mtDNA. Details on the primers, PCR amplification and raw data analyses are given in the Supporting information. MtDNA haplotypes were available for all genotyped individuals as well as one additional male with a unique mtDNA haplotype but no autosomal genotype.

Statistical analyses

We carried out the following analyses: (i) mtDNA diversity patterns, (ii) spatial distribution of females, whose ranging can be followed, (iii) parentage-based pedigree reconstruction and (iv) relatedness estimates. Unless specified otherwise, the analyses included only adult individuals who had potentially already settled within the natal area or dispersed to breed (Prugnolle & de Meeus 2002; Lawson Handley & Perrin 2007), the potential postdispersal (PPD) individuals. We considered individuals as PPD if they were sexually mature and/or regularly seen to range independently from the mother from the beginning of the study period (i.e. ranging at more than 50 m distance for at least several consecutive days). Individuals maturing during the study period were not included as PPD. These criteria resulted in a total of 40 PPD individuals ($n_{females}$ = 15; n_{males} = 25). The number of individuals included in each of the analyses detailed later is summarized in Table S3.

MtDNA diversity patterns and spatial distribution. Using the HVRI haplotypes, we conducted several analyses to assess patterns of mtDNA diversity and lineage relatedness. First, we compared levels of nucleotide and haplotype diversity for the PPD females and males using DNAsp v.5.0 (Librado & Rozas 2009). We tested for a significant difference in haplotype diversity between the sexes using a randomization test. For this, we randomly assigned all observed haplotypes to all males and females 1000 times and counted the number of instances in which the difference between male and female haplotype diversity exceeded the observed one. To show the mutational distances between the haplotypes found in the population as well as their frequencies according to sex, we generated a median-joining network using Network v4.6 and Network Publisher v1.2.0 (Bandelt *et al.* 1999; http://www.fluxus-engineering.com). Second, we assigned individuals to mtDNA lineages, defining these on the basis of haplotype sharing, irrespective of the biparental kinship of individuals.

For the PPD females, we also investigated the spatial distribution of mtDNA lineages using ArcGIS v.9.3.1 (ESRI 2008). To illustrate the areas within the study site where females with the same mtDNA haplotype, i.e. mtDNA lineages, were observed, we used the HRT plug-in for ArcGIS (Rodgers *et al.* 2007) to calculate 95% kernel probability plots, aggregating spatial data for all females with the same haplotype. Hence, incomplete ranging data for the females who also frequently moved outside of the study area did not affect the analyses. Spatial data were available for 13 PPD females (see Supporting information).

Parentage-based pedigree analyses. We examined the precise genetic relationships of female–female, male–male and female–male dyads through a combination of parentage and mtDNA analyses. First, we used the likelihood-based approach as implemented in Cervus 3.0 (Kalinowski *et al.* 2007) to carry out a parentage analysis for all PPD individuals for which data for 24 microsatellite markers were available ($n_{females}$ = 15; n_{males} = 17), as well as nine dependent offspring (see Supporting information).

Simulations were conducted to determine critical values of the log-likelihood score for a 95% confidence parentage assignment. The parameters for these simulations were 10,000 cycles and a minimum of 10 loci typed. The specified genotyping error rate of 0.112% was determined through the 'repeat-genotyping' and 'unintentionally re-sampled individuals' approaches described by Hoffman & Amos (2005). Only PPDs were incorporated as candidate mothers or fathers. The proportion of candidate parents was difficult to estimate from field data. Given the large influence this may have on the statistical significance of the results (Krützen *et al.* 2004a), several conservative values for this parameter (0.05, 0.08 and 0.10) were tested to check the robusticity of assignments. To examine the genetic relationships among all individuals including the seven additional males for which only a panel of six microsatellite markers was available, we repeated the parentage analyses with the same parameters, but with a specification of a minimum of five loci typed.

Following the parentage assignments, we inferred maternal and paternal sibling relationships by examining the shared mothers and shared fathers for each individual in the data set (n = 48). Such a parentage-based pedigree reconstruction allowed assessment of the number of maternal and paternal relatives at the site for each individual, incorporating parent-offspring and sibling relationships. These numbers represent only a minimum bound because the inference of genealogical relationships requires assignment to a parent and hence sampling of this parent within the study site, which may be limited by factors including emigration or death.

Relatedness analyses. We estimated average pairwise relatedness (r) coefficients for all PPD males and females in the data set. Two analyses were carried out. First, r was estimated for all same-sex individuals. Second, r was estimated for each set of same-sex individuals sharing their mtDNA haplotype.

To calculate r estimates, we used the triadic likelihood estimator (TrioML; Wang 2007). This estimator computes relatedness of a dyad in relation to a third reference individual in order to reduce errors stemming from identity-in-state rather than identity-by-descent. It further allows the specification of a genotyping error rate and is bounded between 0 and 1, a more legitimate range than that of other estimators. Moreover, an evaluation using empirical and simulated data for seven different estimators showed that the TrioML produced overall the most accurate estimates (Wang 2007). All PPD individuals were used as the reference population for the background allele frequency calculation. We compared the average relatedness between female dyads and male dyads and tested for significance through 1000 bootstrap re-samplings of the individuals from the observed data set and comparison of the differences in the observed and re-sampled data sets. To show deviations from the population mean, the r estimates were corrected by calibrating the population mean to zero.

In addition, and for comparative purposes, r estimates were also computed with three other estimators: (i) the coefficient of Queller & Goodnight (1989), which is frequently used in the literature and (ii) the coefficients of Wang (2002) and (iii) Lynch & Li (Lynch 1988; Li *et al.* 1993) chosen on the basis of their performance in an estimator evaluation conducted as detailed in the Materials and methods and Supporting information.

Results

Statistical analyses

MtDNA diversity patterns and spatial distribution

We investigated mtDNA diversity and haplotype-sharing patterns. In total, we found 10 different mtDNA haplotypes in Tuanan (Fig. 1; see Supporting information), with an overall, haplotype diversity h of 0.66 (SD ± 0.081) and nucleotide diversity π of 0.006 (SD ± 0.002). Two haplotypes were specific to females: haplotype B was found in four females (10% of individuals) and haplotype C in two females (5%). Another haplotype (A) was very common, found in 23 individuals, and shared by both males (35%) and females (22.5%). The other five haplotypes were all male-specific: haplotype D was present in three males (7.5%), haplotypes E and I in two males each (5%), and haplotypes F, G, H and J in one male each. Interestingly, two of the rare haplotypes unique to the males differed by at least nine mutational steps from the other haplotypes (Fig. 1). The mtDNA variation between the sexes led to a ten-fold higher mtDNA nucleotide diversity in males (π = 0.01 ± 0.002) compared to females (π = 0.001 ± 0.0003). The randomization procedure revealed a significantly higher haplotype diversity in PPD males compared to PPD females (Δ obs ($h_{m/f}$) = 0.102, P = 0.008). Both the presence of sex-specific haplotypes and the significantly higher haplotype diversity in males compared to females are consistent with the genetic predictions for female philopatry and male-biased dispersal.

We were also able to examine the spatial distribution of females, since their home ranges are smaller than those of males and than the study area. While females with haplotypes B and C have their home ranges mainly within the study site, the females with haplotype A range partly in the periphery. Nonetheless, the analyses show

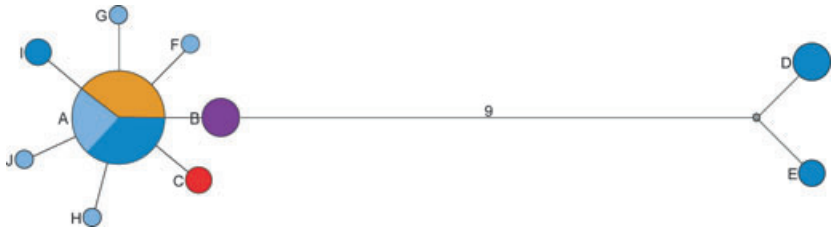

Fig. 1 MtDNA haplotypes in Tuanan. A median joining network of mtDNA haplotypes found in Tuanan is shown. Each different haplotype, shown as a circle, is coloured to represent the proportion of individuals sharing a haplotype: dark blue (flanged males), light blue (unflanged males), other colours (females). Number of mutations between haplotypes is one unless specified.

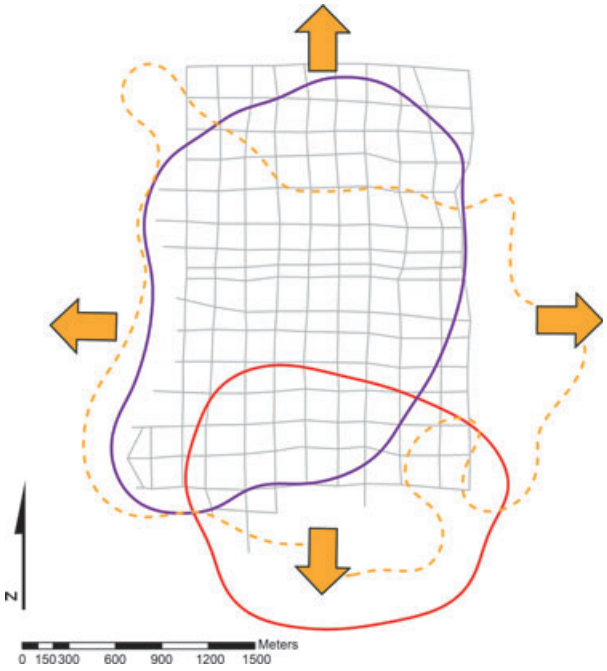

Fig. 2 Spatial distribution of mtDNA lineages in Tuanan. The grid represents the study site, with the combined ranges of females with the same haplotype represented by lines, colour-coded following Fig. 1. The dashed line corresponds to females that frequently moved out of study area (as highlighted by the arrows).

that, within the study site, there is extensive overlap of different mtDNA lineages, indicating that females with different haplotypes share space (Fig. 2).

Parentage-based pedigree analyses

Through the reconstruction of parentage-based pedigrees, we were able to examine the distribution of maternal and paternal relatives among females and males (Table 1). All maternal relationships were confirmed by the observed haplotype sharing. We found that 10 of 15 PPD females had a mother or a PPD daughter at the study site, while only 1 of 24 PPD males was assigned a mother, supporting a model of female philopatry and male-biased dispersal. Particularly the females ranging fully or largely within the study area, those with haplotypes B and C, formed clusters of related individuals. Our results indicate that cluster B comprises a mother and her three adult daughters, two of which in turn have adolescent female offspring. The two PPD females of cluster C were confirmed as a mother–daughter pair. Among the nine PPD females of cluster A, most of which range partly in the periphery of the study site, two mother–daughter pairs were found. The only PPD female with haplotype A and a home range mainly within the study area was not found to have PPD relatives in the area. Field observations indicate that this female had gradually moved from the disturbed habitat in which she had formerly ranged and was consistently chased away at every encounter with other PPD females. None of the males shared haplotypes with the well-known females from clusters B and C, indicating that this is not their natal area. No fathers or paternal relatives were assigned to any of the PPD females or males, indicating that the fathers of adult individuals are not likely to be in the study area.

Relatedness analyses

The average pairwise relatedness estimate *r* as computed with the TrioML estimator was significantly higher among females than males (P value <0.05; Fig. 3). This result was independent of the estimator used, as observed in the comparison across estimators (Fig. S1, Supporting information). We also estimated biparental relatedness for same-sex individuals from the same mtDNA lineage using the TrioML estimator (Fig. 3). The *r* estimates for females with the same mtDNA haplotype were higher than those obtained when all females were pooled together. Males sharing an mtDNA haplotype, by contrast, did not show higher biparental relatedness than all males, irrespective of haplotype. Among individuals with haplotype A, relatedness among females was also significantly higher than that among males.

Discussion

We integrated spatial, observational and genetic data to investigate the dispersal pattern in a nongregarious

Table 1 Maternal and paternal relatives of females and males at Tuanan

		With maternal relatives				With paternal relatives			
Sex	*N*	Mother (%)	Daughter/Son (%)	Sister (%)	Brother (%)	Father (%)	Daughter/Son (%)	Sister (%)	Brother (%)
Females	15	6 (40)	4 (27)	3 (20)	1 (6)	0 (0)	–	0 (0)	0 (0)
Males	24*	1 (4)	–	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

*For seven of the males, autosomal genotypes were available for the six loci used in the identity analyses, determined to be powerful for parentage assignments in assessments of marker informativeness (see Supporting Information).

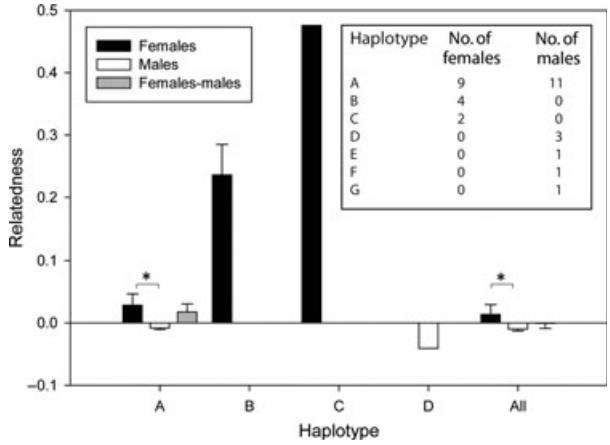

Fig. 3 Female and male biparental relatedness. Trio ML relatedness estimates corrected for population average, as well as variances (error bars) are shown for: all same-sex individuals, and same-sex individuals sharing an mtDNA haplotype. The statistically significant differences in relatedness (P-value < 0.05) are represented by asterisks. For each haplotype, the number of PPD females and PPD males for which complete autosomal genotypes were obtained (microsatellite markers) is detailed in the embedded table.

mammal for which previous genetic studies had produced mixed results. We tested relatedness patterns among individuals through three conventional genetic analyses: mtDNA diversity and haplotype-sharing analyses, the reconstruction of genealogical relationships through parentage analyses and average biparental relatedness. Our results revealed the presence of sex-specific haplotypes and significantly higher mtDNA diversity among males compared to females, underscoring the divergent maternal ancestries of the males. The average pairwise relatedness estimate was higher for females than for males. More importantly, while two-thirds of all females in our study had maternal relatives, with only one case this was the exception for males, indicating a pronounced pattern of female philopatry and male-biased dispersal.

Female philopatry and male-biased dispersal: evidence and comparisons

Among the females, we found three different mtDNA lineages containing clusters of close maternal relatives. For the females with haplotypes B and C, whose home ranges were mainly within the study site, we were able to fully disentangle maternal relationships. Although the relationships among females with haplotype A, most of which range peripherally, are less complete, we did detect two PPD mother–daughter pairs that ranged in the periphery of the study area.

While the female philopatric tendencies supported by our results are congruent with the dispersal patterns in some other solitarily foraging primates, some marked differences are apparent. Notably, there is extensive overlap in home ranges among these females, resulting in spatially stacked matrilineal clusters. These stacked matrilineal clusters contrast with the more spatially distributed maternal lineages in, for example, Coquerel's dwarf lemurs. For this species, Kappeler *et al.* (2002) showed that the sighting centres of females from the same mtDNA lineage are closer than those of females from different lineages. Moreover, within orang-utan clusters, we provided evidence that females are mainly first and second-degree relatives, comprising families of adult mothers and their adult daughters as well as their offspring, while the precise genealogical relationships of females in other nongregarious species are often not known or taken into account, although they may affect nepotistic interactions.

The males in this study, however, differed from the females in several ways. First, they had a far higher diversity of mtDNA haplotypes, most of which were sex-specific. The seven rare haplotypes pertaining exclusively to males highlight their different maternal ancestry compared to the females. This pattern of male-specific haplotypes mirrors the results of studies in the grey mouse lemur and Coquerel's dwarf lemur (Kappeler *et al.* 2002; Wimmer *et al.* 2002; Fredsted *et al.* 2004). Second, males rarely had first-degree relatives in the study area. It was especially revealing that males did not have any mothers or maternal sisters in the study area, except in the case of one young, probably predispersal, male. As none of the males shared maternal ancestry with the well-known centrally located females from clusters B and C, our results indicate that the study site is not a natal area for any of the males. In addition, data on the number of new distinct individuals identified each year indicate that new males keep coming into the study site, while the females are limited in number and well-known after a few years (Fig. S3, Supporting information).

Together, our results match the predictions for a model of female philopatry and male-biased dispersal, in line with previous studies of historical gene flow patterns (Arora *et al.* 2010; Nater *et al.* 2011) and behavioural observation at several orang-utan research sites (Galdikas 1985a; Mitani 1989; van Schaik & van Hooff 1996; Delgado & Van Schaik 2000). Our findings also agree with a recent broad-scale study comparing mitochondrial and Y-linked genetic markers, which provided evidence that orang-utan males move much further than females (Nietlisbach *et al.* in press).

Nevertheless, the patterns we found do not dismiss possible variation in the distances travelled by males, nor a potential range expansion. Some males shared the common haplotype A with the females ranging partly

outside the study area. Thus, it is possible that, unless haplotype A is extremely widespread in the population, these males have their maternal relatives not too far from the study area, suggesting that they have travelled short distances. As male ranges are large and surpass the size of the study site, it is not fully clear whether the males with haplotype A have home ranges that include their natal area, and if so, whether this feature is permanent or temporary, i.e. restricted to early stages of dispersal. Thus, there is a possibility that males with the common haplotype A have expanded their natal ranges, as occurs for instance with bottlenose dolphins (Krützen *et al.* 2004b).

In some cases, males shared their mtDNA haplotype with each other and thus could be maternally related, despite the negative r for males sharing a haplotype as compared to the population mean. Because a parentage-based pedigree reconstruction requires sampling the shared mother to make inferences on shared sibship, inferences on their genealogical relationships cannot be made. However, even if these males were maternally related, parallel male dispersal is unlikely given low male sociality (Delgado & Van Schaik 2000; Utami Atmoko *et al.* 2009). It is nonetheless possible for related males sharing maternal ancestry to converge at a site if the dispersal options are limited because of forest fragmentation and other ecological barriers. This is unlikely to hold for Tuanan, but may be an important consideration elsewhere.

Another interesting finding was that some of the rare sex-specific mtDNA haplotypes were found among unflanged males, who have not yet developed the irreversible secondary sexual characteristics found in the generally older flanged males (Delgado & Van Schaik 2000; Utami *et al.* 2002). Thus, in contrast to suggestions by Morrogh-Bernard *et al.* (2011), our findings indicate that male dispersal may occur when individuals are still young.

Factors affecting the power to disentangle dispersal patterns

Our investigation highlights the importance of several factors affecting the sensitivity of genetic approaches to measure dispersal, particularly for nongregarious species: sampling regime, life history traits and the spatial distribution of individuals.

First, we were better able to resolve the pedigree of females whose home ranges were fully or largely within the study site (cluster B and C), compared to that of females who only partially ranged within it (cluster A). This finding points to the critical importance of size of the sampling area relative to home range size, particularly in nongregarious species. While group-living species have cohesive distinct units of regularly interacting individuals that determine which individuals are sampled, the absence of such units in nongregarious species means that sampling is opportunistic, i.e. spatial rather than group-based criteria, resulting in potential discrepancies between behavioural and genetic results. Especially, the widely used average biparental relatedness estimates are subject to biases stemming from such opportunistic sampling. Species with relatively small home range sizes and small dispersal distances, relative to the sampling area, allow researchers to incorporate larger sample sizes. However, such a sampling regime might lead to the inclusion of unrelated members of the philopatric sex, resulting in lower r estimates than expected. One solution in this case is to measure genetic relatedness against spatial distance (Prugnolle & de Meeus 2002), as has also been performed for various group-living species (i.e. red deer, Nussey *et al.* 2005). To date, studies of a number of nongregarious small-distance travelling mammals show, in agreement with patterns of female philopatry, the expected decrease in female r estimates with increasing geographical distance, and little or no distance effect for males (Coquerel's dwarf lemurs; Kappeler *et al.* 2002; raccoons; Ratnayeke *et al.* 2002; Quail ridge woodrats; McEachern *et al.* 2007). Nevertheless, this approach is not always possible, especially for species with relatively large home ranges and large dispersal distances. Including individuals whose home ranges are not fully encompassed within a study area will reduce the genetic power to detect philopatry if these individuals have their relatives elsewhere.

Second, the slow life histories of some species such as orang-utans and other great apes lead to small sets of closely related individuals at a given time. Thus, in contrast to species with faster life histories, a given sampling area may contain lower numbers of related individuals among the philopatric sex, depending on home range size. It is to be expected then that r estimates decrease with increasing numbers of individuals included in an analysis, as observed in a study of chimpanzees (Lukas *et al.* 2005). Levels of relatedness will also vary depending on reproductive skew, with higher coancestry among the offspring sired by a male with high mating monopolization for instance (Chesser 1991a).

Third, we found stacked matrilineal clusters of females, whose home ranges overlapped. This spatio-genetic structure among females makes it difficult to assess relatedness, as there are both closely related dyads as well as unrelated dyads sharing the same area. This may have been a confounding factor in previous genetic studies of orang-utans, as estimates of average relatedness alone are poor measures of female philopatry. Such spatio-genetic structuring could also

explain cases in other species where, despite behavioural and genetic evidence for female philopatry, average relatedness for females is not higher than expected by chance, as in a study of cougars (Biek *et al.* 2006).

Nonetheless, there may still be some differences in the dispersal patterns across orang-utan populations as a result of intra-specific variation. Such variation would be indicative of facultative dispersal and a high degree of flexibility dependant on population density, local mate and resource competition, and in some cases kin cooperation. In the dusky-footed woodrat, for example, evidence for female kin structures was strongest at intermediate population densities, leading the authors to propose that 'high densities erode kin structures in response to local competition' (McEachern *et al.* 2007). In the grey mouse lemur, despite female philopatry, there is also evidence for the occasional dispersal of females. This was suggested by the spatial conglomeration of females with diverse haplotypes and no obvious female structuring, as well as the presence of multiple clusters of females that were not in spatial proximity but shared the same haplotype (Fredsted *et al.* 2004). In chimpanzees, despite the general pattern of extreme male philopatry and female-biased dispersal, recent research shows great variation in *r* across sites as well as in time (Mitani *et al.* 2002; Nishida *et al.* 2003; Lukas *et al.* 2005). Whereas at sites such as Mahale and Taï, almost all young females emigrate, at Gombe only 50% do and at Bossou none at all. The difference at the latter two sites has been attributed to their lower population sizes and greater isolation from other sites (Mitani *et al.* 2002; Nishida *et al.* 2003). In gorillas, males can either remain in the natal group or leave, and the fitness consequences of dispersal decisions for males at least have been shown to depend partly on demographic variables (Robbins & Robbins 2005). Another interesting possibility is that the recent availability of suitable unsettled habitat, as sometimes accompanies spatial expansions, could change the benefits and costs of dispersal, for instance, by increasing the fitness of dispersers.

Matrilineally related kin structures in orang-utans might confer social benefits to females. Despite the low levels of sociality displayed by orang-utans, associations do occur and have been shown to be more likely among related than unrelated females (van Noordwijk *et al.* 2012). Such associations provide opportunities for play among the offspring of closely related females (van Noordwijk *et al.* 2012). Taken together, these findings support suggestions by Singleton & van Schaik (2002) for the role of nepotistic tolerance in determining the nature of social interactions and opportunities to acquire new skills. Nepotistic tolerance might also make settlement in overlapping home ranges easier for relatives than nonrelatives. Given these results, kin selection may be an important evolutionary mechanism underpinning matrilineal kin structures not only in orang-utans but also in other nongregarious species where these structures remain underexplored, and which warrant detailed investigation.

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N.A., C.A., A.N., M.G., P.N., and M.K. apply genetic methods to study evolutionary, population genetic and ecological questions in wild animal populations. M.K. and E.P.W. investigate the evolution of culture and cooperation using genetic and spatial data. S.S.U.A., J.P., and D.P.W. study Indonesian primates. L.D., Mv.N. and Cv.S. are interested in the social evolution and cognition of primates.

Data accessibility

The mtDNA HVRI sequences have been deposited in EMBL under accession numbers FR717918–FR717919 and FR717921–FR717924. The genotypes and haplotypes for each individual, as well as the spatial distribution data for the females, are provided in the Supporting information.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Supporting Information Methods and Results.

Appendix S2 Supporting Information Tables and Figures.

Appendix S3 Supporting Information Genotypes and Haplotypes.

Appendix S4 Supporting Information Haplotypes.

Appendix S5 Supporting Information nexus file Tuanan New Haplotypes.

Appendix S6 Supporting Information Ranging Data.

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ORIGINAL PAPER

Female philopatry and its social benefits among Bornean orangutans

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Abstract Female philopatry in mammals is generally associated with ecological and sometimes social benefits, and often with dispersal by males. Previous studies on dispersal patterns of orangutans, largely non-gregarious Asian great apes, have yielded conflicting results. Based on 7 years of observational data and mitochondrial and nuclear DNA analyses on fecal samples of 41 adult Bornean orangutans (*Pongo pygmaeus wurmbii*) from the Tuanan population, we provide both genetic and behavioral evidence for male dispersal and female philopatry. Although maternally related adult female dyads showed similar home-range overlap as unrelated dyads, females spent much more time in association with known maternal relatives than with other females. While in association, offspring of maternally related females frequently engaged in social play, whereas mothers actively prevented this during encounters with unrelated mothers, suggesting that unrelated females may pose a threat to infants. Having trustworthy neighbors may therefore be a social benefit of philopatry that may be common among solitary mammals, thus reinforcing female philopatric tendencies in such species. The results also illustrate the diversity in dispersal patterns found within the great-ape lineage.

Keywords Male dispersal · Female philopatry · Pedigree · Female–female association · Range overlap · Social play

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Introduction

In most mammals, females show philopatry, i.e. settle for life in or near the area in which they are born, whereas males disperse (Greenwood 1980; Waser and Jones 1983; Pusey and Packer 1987; Lawson Handley and Perrin 2007). Philopatry has ecological advantages in that it enables the individual to continue to live in a familiar habitat and maintain a familiar diet, without having to experience the costs in terms of time, travel, and risk of finding a suitable area in which to settle. In addition, philopatry may enable individuals to maintain lifelong supportive social bonds with known relatives. In group-living primates, for instance, philopatric females tend to live in close association with their maternal relatives, who may provide mutual support in conflicts with less-related group members (e.g., Silk et al. 2009; 2010; see also Holekamp et al. 2012 for a similar system in spotted hyenas). Likewise, elephants living in fission–fusion female groups maintain supportive social relationships and show preferential associations with their matrilineal relatives in or near their natal range (De Villiers and Kok 1997; Archie et al. 2006). Thus, philopatry may bring social as well as ecological benefits. On the other hand, philopatry may limit access to unrelated and willing mates. Thus, in general, if members of one sex derive a clear advantage from being

(more) philopatric, the other sex tends to derive a reproductive benefit from moving further away (Bengtsson 1978).

For species with strong ecological pressure on females to be more or less solitary, the social advantage of their philopatry is likely to be reduced, and therefore the tendency for female philopatry may be relaxed. Nonetheless, genetic and radio-telemetric research has confirmed that females in many non-gregarious mammal species settle in home ranges adjacent to, or overlapping with, their natal range and thus their female kin (e.g., tigers, Smith 1993; raccoons, Ratnayeke et al. 2002; bears, Støen et al. 2005; Moyer et al. 2006; woodrats, McEachern et al. 2007).

One possible explanation for the philopatric tendency of females in solitary mammals is that the ecological benefits on their own are strong enough to favor female philopatry, especially in species where males have much larger ranges than females and do not derive reproductive benefits from remaining philopatric. Where males derive strong benefits from philopatry, for example through coalitionary defense of a range or access to females, the less gregarious females are likely to disperse (e.g., chimpanzees, Langergraber et al. 2007; spider monkeys, Di Fiore et al. 2009). However, it is also possible that "solitary" females in many species do accumulate social benefits, which accrue at rare but critical times in the life cycle. Indeed, there is evidence that, in some solitarily foraging species, females may be temporarily gregarious when they have dependent offspring. For example, females of the nocturnal mouse-lemur, *Microcebus murinus*, may share a nest hole for their young and even allo-nurse each other's young. Only closely related females have been observed to share in this way, thus both gaining foraging efficiency and maybe increased survival of their offspring through better thermoregulation and increased chances of adoption (Eberle and Kappeler 2006; cf. König 2006 for house mice). Some bats also associate at the roost preferentially with close maternal relatives during lactation, mutually gaining thermoregulatory benefits during a period of high energetic demands (Kerth et al. 2002). These observations raise the question whether social benefits are more common than usually assumed, which would strengthen a female philopatric tendency even in largely solitary species. Unfortunately, for most species it is unclear whether and how females interact differently with their related neighbors compared to unrelated ones, and thus whether they derive social benefits from philopatry at some stage during their lives.

Sumatran (*Pongo pygmaeus*) and Bornean (*Pongo abelli*) orangutans are the only ape species in which both males and females are habitually non-gregarious, like in many non-primate mammals. Especially the Bornean species shows a strong tendency towards solitary life (van Schaik 1999; van Noordwijk et al. 2009). This solitary lifestyle suggests that advantages for either sex of philopatry would be limited to the ecological advantage of familiar range and diet, unless unexpected social benefits are present. For this reason it is interesting to assess the degree to which females or males remain philopatric, and relate these tendencies to a detailed study of their social behavior.

Another reason for interest in orangutan philopatry patterns is that they are one of our closest living relatives. In chimpanzees, males are found to be the more gregarious sex and to be strongly philopatric, allowing them to form long-lasting bonds with their relatives, whereas females tend to disperse from their natal community (Langergraber et al. 2007). In another close relative, the gorilla, females as well as males usually disperse (e.g., Douadi et al. 2007; Robbins et al. 2009), whereas males in Eastern gorillas males may occasionally "inherit" the groups in which they are born (Watts 2000; Bradley et al. 2007). Thus among the extant African apes at least female-biased dispersal seems to be the dominant pattern and has been proposed to be the tendency in the last common ancestor of humans and African apes (Ghiglieri 1987). Although various authors have assumed that female dispersal is a deeply rooted hominoid tendency (e.g., Foley and Lee 1991; Hrdy 2009; Chapais 2010), the "natural" human dispersal system has remained a topic of debate (Hill et al. 2011). Therefore, establishing the natural dispersal pattern of the orangutan may provide a broader perspective to the reconstruction of the origins of the various dispersal patterns found within the hominoid lineage.

Long-term field observations on orangutan populations on both Sumatra and Borneo indicate that adult males have very large overlapping ranges, but rarely associate with each other (Galdikas 1985; van Schaik 1999; Utami Atmoko et al. 2009), and concentrate their associations with females largely to the periods in which these are potentially fertile. There is no evidence for male social bonds, as in chimpanzees, and thus male philopatry is not expected. Females have smaller ranges than males (Singleton et al. 2009; Utami Atmoko et al. 2009), which can also largely overlap, and they spend most of their time accompanied only by their dependent (and sometimes one semi-dependent) offspring (van Noordwijk et al. 2009). For Sumatran orangutans, Singleton and van Schaik (2001; 2002) found clusters of females who associated more often with each other than expected based on their (extensive) range overlap. If females from the same cluster met, they were also more tolerant of close proximity than when females of different clusters encountered each other. These authors hypothesized that such female clusters were based on relatedness, thus implying female philopatry with some social benefits, but lacked the genetic data to verify this. On the other hand, Knott et al. (2008) emphasized for a Bornean population (Gunung Palung) that females show active avoidance of other females within their overlapping ranges, even though assumed relatives had more frequent encounters. In addition, they

concluded that the outcome of a female-female encounter depended on its location relative to the females' respective core areas and thus that females defended their ranges. Thus, although female philopatry may be present in both orangutan species, social benefits appeared to be modest at best among Bornean orangutans.

Behavioral studies on maturing individuals have suggested a tendency towards female philopatry and male dispersal in orangutans of both islands (van Schaik and van Hooff 1996). However, the first studies that estimated genetic relatedness patterns among males and females in several populations have reached divergent conclusions, ranging from both sexes dispersing equally (Utami et al. 2002) to being equally philopatric (Goossens et al. 2006) to male-biased dispersal with female philopatry (Morrogh-Bernard et al. 2011). Nevertheless, recent population genetic analyses using both mitochondrial DNA and Y-chromosome markers indicate a spatial structuring congruent with male dispersal over large distances and very restricted female dispersal for both species (Arora et al. 2010; Nater et al. 2011; Nietlisbach 2009).

The aim of the present study was to use a combination of genetic, ranging and socio-behavioral data of individuals in an intensively studied population of orangutans (*Pongo pygmaeus wurmbii*) in Tuanan (Central Kalimantan, Indonesia) to assess for this population (a) whether females and/or males are philopatric or disperse and (b) whether philopatry was accompanied by social advantages. The genetic analyses focused on detecting mother–adult offspring dyads based on mitochondrial and nuclear DNA (Arora et al. unpublished data). Given the stability of adult females' ranges (e.g., Wartmann et al. 2010 for the same population), the mother's range is the best estimate of any adult individual's natal range and thus maternal relatedness among adults is essential for documenting dispersal patterns.

Materials and methods

Study population

The Tuanan Orangutan Research Area (2° 09′ South; 114° 26′ East) is located in formerly selectively logged swamp forest on shallow peat (<2 m) in the Mawas Conservation Area, Central Kalimantan, Indonesia. The orangutan population here has been studied since June 2003 and has approximately 4.5 individuals per square kilometer (van Schaik et al. 2005). Individuals born before the start of the observations were assigned to age–sex categories based on prior experience (cf. Wich et al. 2004). The study area of ca. 750 ha (gradually enlarged to >1,000 ha) encompassed several complete ranges of adult females, but ranges of all known males extended beyond this limited area. In this population, adult females spent on average less than 20% of their time in association with another adult conspecific (van Noordwijk et al. 2009).

Observations in the field followed the standardized orangutan protocol (see www.aim.uzh.ch/orangutannetwork). Analyses on adult female ranging, association pattern, activities and social interactions are based on more than 16,650 h of focal observation on 8 different mothers collected by a well-trained team of observers from July 2003 to July 2010. Most data were collected during nest-to-nest follows lasting a maximum of 10 consecutive days per month per focal female. During nest-to-nest follows, observation time was counted from the moment the focal female left her night nest in the morning until she rested in a (new) night nest in the evening (average active time per day for adult females was 10 h 50 min; N=1,330).

Adult female home ranges were found to be highly stable over time (Wartmann et al. 2010). Here we included data on adult females (who had at least 1 offspring and were habituated to human observers) if in total at least 250 h of focal observation and ranging data were collected for that female (thus excluding 5 known adult females ranging at the periphery of the study area). For each dyad of females, only those data collected during the same years were used for a dyad-specific sample (resulting in different sample sizes per dyad—see supplemental Table 1). All but one focal female had a dependent unweaned offspring (i.e. <7 years old) for at least part of the data collection period, and three focal females were accompanied at least part of the time by a young infant as well as a weaned offspring. In total the regular focal females had five female and four male immature offspring.

Sightings of all identified individuals were recorded for each month throughout the study period. The percentage of months an individual was present in the study area was based on focal follows as well as sightings during focal follows of other individuals or other research activities in the forest. Observational data on males were collected in the same way as for females. In total, over 10,500 h of focal data was collected on those males sighted during at least 10% of the observation months. Data for the two morphs of adult males are presented separately, i.e., for flanged (with full secondary sexual characteristics, including cheek flanges) and unflanged males (without such characteristics, but capable of siring offspring under natural conditions: Utami et al. 2002; Goossens et al. 2006). All young adult males are unflanged, but the age at which an individual male develops flanges is probably highly variable (Utami Atmoko et al. 2009).

Genetic analyses

We evaluated the maternal relatedness of individuals at the study site through maternity analyses and inference of

maternal siblings, using biparentally inherited microsatellite markers and a maternally transmitted mitochondrial DNA (mtDNA) marker. Fecal sample collection and storage was carried out using a standard genetic sampling protocol http://www.aim.uzh.ch/orangutannetwork/GeneticSamplingProtocol.html, followed by the generation of data on autosomal microsatellites and mitochondrial DNA (mtDNA; details in Arora et al. 2010). We verified that the samples belonged to distinct individuals by genotyping them at six microsatellite markers, which had a combined non-exclusion probability of 1.36×10^{-5} and 8.90×10^{-3} for unrelated individuals and full siblings, respectively, as calculated in Cervus 3.0 (Kalinowski et al. 2007). This procedure identified 47 unique individuals. For 41 of these individuals we generated genotypes at an additional panel of 18 loci, totaling 24 autosomal microsatellite markers. For six males low DNA quantity and quality did not allow us to complete the genotyping for all loci. Thus, for these males, only the identification panel of six markers was used, which in combination with the use of mtDNA markers was sufficient to exclude all but one mother–son dyad (Arora et al. unpublished data)

To identify mother–offspring pairs, we carried out maternity analyses using the likelihood approach implemented in Cervus 3.0 (Kalinowski et al. 2007), using the strict 95% confidence level. All individuals were assessed as potential offspring, but only sexually mature females were incorporated as candidate mothers. To determine critical values of the log-likelihood score for a 95% confidence parentage assignment, we ran 10,000 simulations with the following parameters: a minimum of 6 loci typed, and our genotyping error rate of 0.112% (Arora et al. unpublished data) as empirically determined through the "repeat-genotyping" and "unintentionally re-sampled individuals" approaches described by Hoffman and Amos (2005). The proportion of candidate mothers sampled was difficult to estimate from field data. It has been shown that this parameter may have a substantial influence on the statistical significance of the parentage assignments (Krützen et al. 2004). Thus, we tested several conservative values (0.05, 0.08, and 0.10) and corroborated that the results were robust. In all cases, mother–offspring dyads detected using microsatellite data, also shared their mtDNA haplotype. In addition, all known mother–unweaned offspring dyads, with samples available for both ($N=8$), were confirmed to have a genetic mother–offspring relationship according to our procedures. Individuals sharing a mother were inferred to be maternal siblings. A limitation to this approach is that individuals that are maternally related may not be detected due to the absence of (a sample of) a shared mother. However, this bias is not expected to differ for females compared to males. For all further analyses, maternally related dyads were defined as either a mother–offspring pair, or two individuals sharing a mother.

Ranging behavior

The ranging behavior of 8 focal females was investigated at the level of both home range and core range areas to obtain estimates of proportional dyadic overlap. Using locational data collected at 30-min intervals (see criteria for inclusion above), the respective ranging areas of all females were calculated on a dyad-specific basis. We delineated home ranges by 95% volume isopleths (Anderson 1982) on utilization distributions obtained from fixed Gaussian kernel density estimation (using BCV to estimate the kernel's bandwidth; Worton 1989), whereas core areas were defined by 50% volume isopleths. Areas of overlap were subsequently calculated and divided by the dyad-specific home range area of each focal female. This resulted in an asymmetric matrix for both proportional home range and core range overlap of 52 of the 58 possible dyads (insufficient concurrent ranging data were available for 6 dyads). All analyses on ranging behavior were conducted using the HRT plug-in (Rodgers et al. 2007) and Spatial Analyst extension for ArcGIS 9.3 (ESRI 2008).

Association and social interaction analyses

Whenever 2 individuals approached to within 50 m, this was considered an encounter. Encounter rates were based on the number of new approaches within 50 m occurring during a focal follow starting at the morning nest. If a dyad had spent the night within 50 m of each other, the (continuing) association the next morning was not counted as a new encounter. The percentage of time in association (<50 m distance) was based on total active time from the focal female's perspective. The relationship between time in association and range overlap between two females was based only on those years for which ranges for both could be calculated. When individuals were simultaneously feeding in the same food source <10 m apart they were said to show "feeding tolerance." An agonistic interaction was defined as one in which one individual shows clear aggressive acts such as slapping, grabbing, biting or a fast chase and/or the other shows obvious avoidance or submissive behavior such as fleeing fast (through canopy or over the ground) or screaming. In the analyses, social play among offspring of different mothers could include both unweaned and weaned offspring as long as these were in permanent association with the mother.

Unfortunately, as in previous studies on wild orangutans, the small number of mother–offspring dyads did not allow for an analysis of the effect of offspring sex (van Noordwijk et al. 2009). However, so far, no striking differences between female and male dependent offspring in time budgets or social interest have become apparent.

Statistical analyses

Given that dyadic data are inherently non-independent, permutation versions of standard statistical techniques were employed in which significance of test-statistics was assessed by 10,000 randomizations. To test whether the degree of overlap in ranging areas (both at the home and core range level) differed between related and unrelated female dyads, permutation unpaired *t* tests were conducted (Legendre and Legendre 1998). Differences in the duration of association among dyads were compared in a permutation one-way ANOVA test (Legendre and Legendre 1998). Post hoc pairwise comparisons (Tukey's honestly significant difference tests) subsequently revealed which categories of dyad were significantly different from each other. Potential associations between overlap in ranging areas and encounter frequency and association time were considered separate for unrelated and related female dyads by computing Pearson permutation correlation tests. Differences in the encounter frequencies and duration of association between unrelated and related female dyads were investigated in more detail by a permutation unpaired *t* test.

Results

Genetic analyses

In total, 10 different mtDNA haplotypes were found for the individuals sampled in Tuanan. All females (including all additional adolescent and nulliparous ones) had one of only three haplotypes; whereas for the males, eight different ones were found. Only one haplotype was shared by males and females. This distribution of haplotypes was significantly different for females and males (Arora et al. unpublished data).

Pedigree analysis based on nuclear and mtDNA indicated the presence of one mother with three adult daughters (for which maternal sibship was thus inferred) and one mother–adult daughter dyad, all with ranges mostly inside the study area. In addition, 2 independently ranging adolescent females could be matched with their mothers ranging in the periphery of the study area. In contrast among the 28 males, only 1 young one (estimated 10–15 years old) could be matched with his peripherally ranging mother. Thus, both the much greater concentration of females in a few haplotypes and diversity of haplotypes of the males and the sex difference in the presence of dyads of maternally closely related adults in the study area are consistent with greater female philopatry and male dispersal.

In the following sections we refer to the 4 genetically detected mother–adult daughter dyads as well as the 3 female–female sibling dyads (based on sharing the same mother) as "related dyads" and all other female–female dyads as "unrelated dyads."

Spatial analyses

Male ranges and overlap

None of the known adult males had his complete range within the study area. In addition, most of the known males were seen throughout this area. Thus, male ranges were estimated to be considerably larger than even the expanded study area of 1,000 ha. In addition, all known males were regularly observed to leave the study area. Only 1 (flanged) male was sighted during ca. 50% of the observation months (compared to 4 females during >75% of the months), whereas 13 additional males (7 flanged; 5 unflanged) were sighted during at least 10% of the months (the other 14 genetically identified males [11 flanged and 4 unflanged] were sighted less often). These observations suggest major home range overlap among the many adult males sighted in the area.

Female ranges and overlap

Some females could never be followed for more than a few consecutive days before they left the study area, whereas others never travelled outside of the study area during follows. In the analyses of home range overlap we only included those females for whom we were confident to have adequate data to calculate their 95% and 50% use area, based on 1–8 years of data per dyad. Overlap was always calculated from the perspective of the focal female (resulting in 2 different values per dyad). The average home range size for females with ranges inside the study area was 327.5±124.7 ha, with a core area (50% use) of on average 84.0±27.6 ha.

All known maternally related female dyads had overlapping home ranges (Fig. 1; average HR overlap per dyad: 57.32%, N=12), as well as overlapping core areas (average 15.79% (Fig. 2a). Within the study area, non-related female dyads (N=40) had an average overlap of 36.90% and core range overlap of 6.85%, which is significantly less (home range overlap: permutation *t* test, $t_{(50)}$=2.51, P=0.015, N_{perm}=10,000; Core range overlap: permutation *t* test, $t_{(50)}$=2.25, P=0.027, N_{perm}=10,000). However, more than half of the unrelated dyads (N=23) exhibited a degree of overlap equal to or greater than the minimum observed overlap between related dyads (32.1%), and when comparing these (average 54.04% HR overlap and 10.76% CR overlap) to related dyads, differences were no longer significant; permutation *t* test for HR overlap: $t_{(33)}$=0.466, P=0.65; CR overlap $t_{(33)}$=1.029, P=0.32). Thus matrilineal clusters of females have overlapping ranges, but home ranges and core areas may be shared with unrelated adult females as well, and to a similar extent.

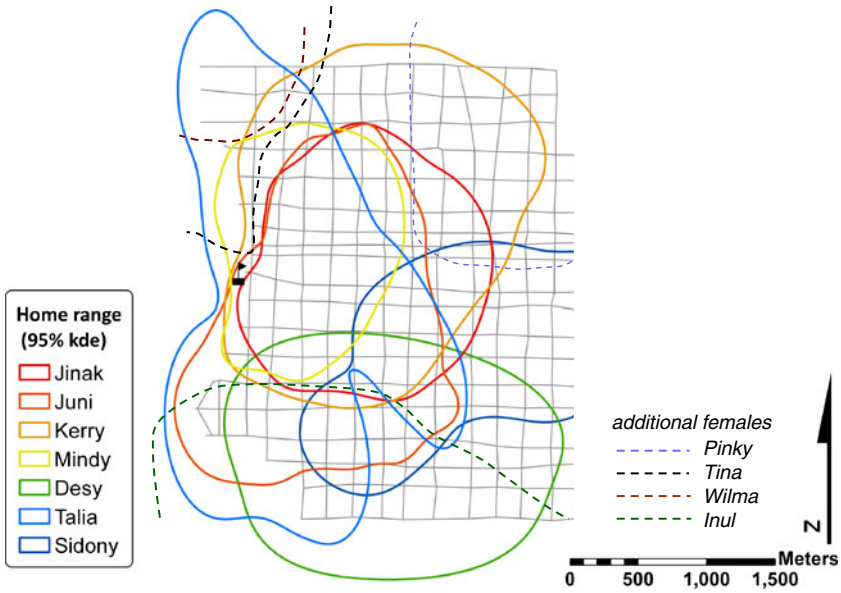

Fig. 1 Map of the overlapping home range areas (95% use) of 7 focal females calculated for 2008. Females Jinak, Juni, Kerry, and Mindy are members of the same matriline; Desy is the daughter of Inul, and for Talia, and Sidony no matrilineal adult female relatives are known. Also indicated are the parts of the home ranges of the additional females Wilma and Tina (probably maternal relatives, but not mother–daughter) and Pinky for as far as they are known in the study area. The research station is indicated with a *flag*. (The area to the west of the study area is burnt and severely degraded habitat)

Social relationships

Male–male social relationships

During 7 years of focal sampling on adult males, male–male encounters and associations were rare. Flanged males spent on average 0.11% of their focal time in association with another flanged male, flanged with unflanged males on average 0.25%, and unflanged with unflanged males 3.12%, but only 1.0% when they were not also in association with an adult female. Associations among male dyads of all combinations lasted significantly shorter than those among closely related females, but were not different from those of unrelated females (permutation one-way ANOVA: F=20.70, P=0.0001; post hoc pairwise comparisons—Tukey's HSD—reported in Supplementary Table 2).

Only unflanged males occasionally showed feeding tolerance towards other unflanged males (at least briefly during 26% of unflanged male associations, or 8% of all male–male associations). Social play among two unflanged males was observed during five associations—(four times involving the same male with three different partners) and except for one event such play lasted only for a few minutes. No grooming or coalitions against another individual were ever observed among males and only one association between unflanged males was continued the next morning (see also Supplementary Fig. 1). Thus, we found no evidence for any special relationships, social bonds or coordinated ranging behavior among adult males in this population.

Female social relationships

Female encounters and associations The frequency of encounters amongst female dyads was not related to the percentage of home range overlap (permutation correlation test: related dyads: r_{Pearson}=−0.22, N=12, $P_{\text{perm }(n=10,000)}$= 0.49; unrelated dyads: r_{Pearson}=0.19, N=40, $P_{\text{perm }(n=10,000)}$= 0.24). Even degree of core range overlap was not significantly correlated with encounter frequency among related dyads (r_{Pearson}=−0.16, N=12, $P_{\text{perm }(n=10,000)}$=0.62), whereas it was positively correlated for unrelated dyads (r_{Pearson}=0.41, N=40, $P_{\text{perm }(n=10,000)}$=0.019). However, if we only consider those unrelated dyads with a total home range overlap equal to or greater than the minimum observed amongst related females, there was only a trend (r=0.34, N=23, $P_{\text{perm }(n=10,000)}$=0.09).

Overall, females encountered their close maternal relatives more often than other females with similar degree of home range overlap, i.e., those unrelated dyads with an overlap of more than the minimum among related dyads of 32.1% (permutation unpaired t test: t=6.49, N_1=12, N_2=23, P=0.0001). This distinction between relatives and other females is also reflected in the percentage of time focal females spent in association with other adult females (Fig. 2b; t=5.59, N_1=12, N_2=23, P=0.0001). Additional analyses of encounter rate and association duration divided by percentage home range and core range overlap respectively, yielded also very significant differences between related and unrelated dyads (Fig. 2c).

Once associations occurred, their duration among related females was also longer than among non-related females (Supplementary Table 2). In addition, none of the 37 observed associations between unrelated females lasted for more than 3 h and none was maintained overnight, whereas 96 of 194 (49.7%) associations of related females lasted more than 3 h, and in 54 out of 194 (27.8%), a night nest was made within 50 m of each other. This bias in both encounter frequency and association time strongly suggests that spending time in association is an active choice and not a by-product of range overlap.

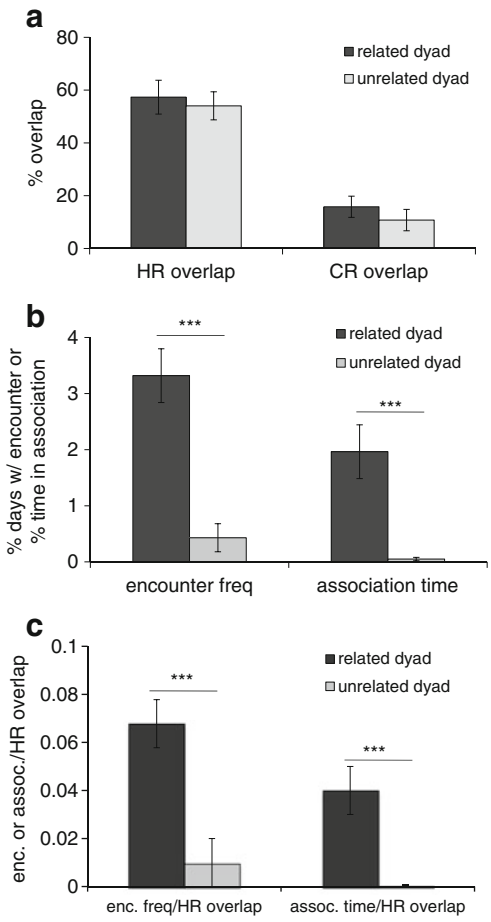

Fig. 2 Comparison between maternally related females dyads and unrelated dyads in (**a**) their home range (95% use area) and core range overlap (50% use area), (**b**) encounter frequency (% of days with an encounter per dyad) and percentage of total focal time spent in association, and (**c**) encounter frequency and association overlap controlled for home range overlap. Unrelated dyads were only included if their home range overlap was at least 32%, the lowest overlap among related females. Significant differences between adjacent columns are indicated with ***$P<0.0001$

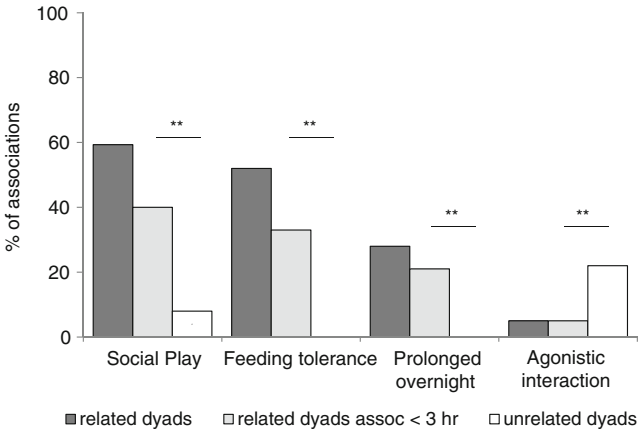

Fig. 3 Comparison of social behavior during associations between related females, associations between related females lasting <3 h and associations between unrelated females (all lasting <3 h): the percentage of female–female associations with at least some social play among immatures, feeding tolerance among females (i.e., both feeding within 10 m), prolongation of the association overnight, agonistic interaction between the females. Significant differences between adjacent columns are indicated with **$P<0.001$

Social interactions during associations Agonism. Even if we restrict analyses to the relatively short associations (<3 h) observed among unrelated females, associations among non-kin dyads differed from those among kin-dyads (Fig. 3), with a higher frequency of severe agonistic interactions (i.e., with active chase and or physical fight including hitting or biting: in 8 out of 37 associations vs. 5 out of 98 Fisher exact $P=0.008$). In addition, agonistic interactions among non-relatives always (8/8) led to immediate termination of the association and only in 2/5 of the cases among related dyads. (All severe agonism among relatives was between the same two half-sisters).

Feeding tolerance. During associations females hardly ever engaged in positive social activities (only once brief grooming in a mother–adult daughter dyad), but sometimes showed tolerance by feeding within 10 m of each other in the same food patch. Such "feeding tolerance" was never seen during associations of non-related dyads, but in 32 of 98 (32.7%) of those of maternal relatives ($\chi^2=14.08$; $P<0.001$; Fig. 3).

Social play. Despite the females' lack of affiliative interactions during most associations, their respective offspring frequently engaged in social play (mostly arboreal play-wrestling). Related mothers rarely terminated, and sometimes even actively enabled play among peers while protecting very young infants, and mothers, especially of infants <3 year of age, sometimes even actively participated in such play. However, mothers intervened and effectively prevented contact among non-kin peers on several occasions, by either retrieving their own offspring or chasing away the other immature if it was accompanied by its mother. As a result, social play between unweaned immatures was only seen (briefly) during 3 (of 37) associations between non-related female dyads, whereas play was observed during 40 (of 98) of the associations between relatives ($\chi^2=11.77$; $P<0.001$; comparing only associations lasting <3 h to decrease bias in favor of related dyads; Fig. 3). Nevertheless, when weaned immatures ranging independently from their own mother (>50 m away) visited a non-related mother–offspring pair, they were "allowed" to play with dependent offspring (observed during at least 6 different focal follows), even for a prolonged period (average play duration 60 min).

In summary, encounters between unrelated females rarely resulted in long-lasting association, and never included simultaneous feeding in the same food patch. Agonistic termination of such associations was also more likely than of associations among related females. In contrast, the close kin associations are characterized by tolerance during feeding and tolerance and accommodation of play interactions among their offspring.

Female cluster size and infant playing time

The Tuanan study area included the ranges of one matrilineal kin-group with a mother and her three adult daughters and another mother–adult daughter dyad ranging at the edge of the study area (insufficient data on one of these two females). In addition, data were collected on three adult females ranging mostly (but not completely) in the study area, and for these females no living adult maternal relatives were known. Even though these females may have had relatives living outside the study area, those were never encountered during follows. Thus in the study area, immatures had access to kin clusters of different sizes, and therefore they were likely to grow up with different opportunities for social interactions with peers. Indeed, in this study females and their offspring of the larger kin cluster with four adult females spent more time in total in association with other adult females than those with only one or no known adult maternal relatives (average 6.15±3.07% vs. 0.34±0.08%; Mann–Whitney $U=0$, $N_1=4$, $N_2=4$, $P<0.05$ two-tailed; whereas large and small kin cluster females did not differ in % time in association with unrelated females: $U=10$, $N_1=4$, $N_2=4$, NS). Even though this comparison is based on only one large maternal cluster *vs*. several females from small clusters, these data suggest a matriline size effect on social opportunities for maturing offspring.

The time difference in time spent in association with peers (and their mothers) was reflected in the time budgets for 1–5-year-old-dependent immatures, indicating that immatures growing up in the large kin cluster consistently spent more time in social play than immatures growing up in a small kin cluster (average 1.18±0.92%, $N=11$ yearly values of at least 150 h of focal data for the large cluster vs. 0.06±0.06%, $N=5$ for the small; Mann–Whitney $U=4$, $N_1=11$, $N_2=5$, $P<0.01$ two-tailed). Despite the small sample sizes, these data strongly suggest that maternal cluster size affects the amount of social play with peers an immature can achieve.

Discussion

Dispersal and philopatry

We used observational and genetic data from a single study area to investigate the relatedness patterns among the resident adults in a non-gregarious great ape. Genetic analyses focused on close maternal relatedness, since female ranges were very stable and the maternal range is therefore the best predictor of an adult's natal range. The combination of ranging data and the genealogical reconstruction of all adults sighted in the study area indicated that whereas 4 parous (plus at least 2 nulliparous) females lived in overlapping ranges with their surviving mother, we found only one young male being maternally related to a known female at the periphery of the study area. Thus, our results strongly suggested female philopatry (sensu Waser and Jones 1983) and male dispersal and are therefore consistent with other recent genetic studies (Arora et al. 2010; Morrogh-Bernard et al. 2011; Nater et al. 2011).

From the Tuanan results, we could also estimate the age at which males disperse from their natal area. The 1 male with a female relative in the study area was still young (estimated <15 years old) and never seen in the same area or in association with his mother. At least 4 unflanged males had a "nonlocal haplotype," e.g., different from all of the sampled parous females (Arora et al. unpublished data). These 4 males were frequently present (10–50% of the months) in the study area, indicating they had moved away from their natal area while they were still unflanged. We therefore conclude that males tend to disperse from their natal range as adolescents. Morrogh-Bernard et al. (2011) concluded that males do not disperse before growing flanges. However, their conclusion was based on a small sample, and is not easily reconciled with the fact that unflanged males are known to sire offspring (Utami et al. 2002; Goossens et al. 2006). We therefore suspect that our conclusion holds more generally.

Recent genetic analyses of the Y-chromosome suggest that males may disperse remarkably long distances away from their natal areas (Nietlisbach 2009). However, here we found that male orangutans not only disperse spatially (away from natal area), they also do so socially (away from known relatives; cf. Isbell and Van Vuren 1996). First, among the males in the study area, no close maternal male kin could be identified. Second, male–male associations, even among unflanged males, were of significantly shorter duration than associations between related females, and no consistent associates could be detected, suggesting the absence of the social bonds expected if there was parallel dispersal (see also Supplementary Fig. 1). Females, in contrast, appear to stay in the familiar area and also near their familiar female relatives, although we would need to sample a larger area to assess whether all females manage to do so. Overall, therefore, the genetic results strongly support behavioral evidence for female philopatry and continuing association with relatives in Bornean orangutans, and the opposite pattern in males.

Female relationships and the social benefits of philopatry

It is possible that maturing individuals with a greater number of tolerant role models would have acquired a larger number of learned skills by the time they are adult. Although infants peer largely toward the activities of their own mothers they also do so occasionally with other cluster

members (Jaeggi et al. 2010), but never with females of other clusters. However, given the small sample sizes, the fitness benefit is hard to quantify. Future work will attempt to estimate these benefits.

In this study, the most conspicuous behavior during associations was social play. Even though related female dyads seemed to choose to approach and spend time in proximity, adults tended to watch each other initially and only gradually approach to within 5 m. Females' dependent offspring, however, tended to approach each other quickly and start social play within the first minutes of the start of the association. Successful maternal intervention, by chasing the other immature or retrieving her own offspring, was seen on several occasions when unrelated females were in brief association and their infants approached each other. The few observations of social play between unrelated immatures almost all happened in the absence (>50 m away) of 1 of the mothers, or when 1 of the mothers was distracted by a consortship with a male. Lack of maternal proximity and attention only occurred for immatures of at least 5 years old. This pattern suggests that the lack of play among peers born in different maternal clusters is due to the mothers' reluctance to allow their offspring to interact and not to a lack of interest on the part of the immatures.

Opportunities for social play among peers are rare for this sample of wild Bornean orangutans. Even though some mothers occasionally actively engaged in social play with their young offspring, they certainly did not do this every day. An older sibling, if present, is at least 7 years older and thus much larger and, most importantly, no longer in frequent association by the time the infant is 2–3 years old and ready to move more than a few meters away from the mother (van Noordwijk et al. 2009). However, when related mother–offspring pairs were in association, immatures frequently played and seemed to forego not only solitary play (which accounts for 20–50% of a 1–4-year-olds' average time budget), but also reduced their time feeding and resting (sometimes by >10%; unpublished data). Even before weaning, social play in this population did not exceed 4% of the average yearly time budget, whereas it drops to less than 1% after weaning (unpublished data). Nevertheless, immatures seem to take advantage of every opportunity they can get to engage in social play with peers, whereas during their associations, adult females rarely engage in social exchanges (such as grooming or food sharing) and merely tolerate proximity (or not). Thus association among parous females seems to be in the interest of the offspring more than of the mothers themselves.

Why would it be beneficial for mothers to provide opportunities for social interactions with peers? Social play, especially during development, is seen in all primates and indeed most mammals and birds as well as other vertebrates (Fagen 1981; Graham and Burghardt 2010). The major functions of play are thought to be facilitation of motor development (e.g., Byers and Walker 1995; Nunes et al. 2004), and brain development (e.g., Lewis and Barton 2006; Pellis 2010), and preparing the individual to respond to unexpected events (Špinka et al. 2001). Yet, the fitness consequences of a lack of social play under natural conditions are still little known and hard to measure, although one study on Alaskan brown bears, (Fagen and Fagen 2009) reported a positive correlation between social play and survival to independence in Alaskan brown bears, irrespective of food availability and maternal condition. However, despite several plausible hypotheses about the benefits of social play, it remains to be determined whether the difference we found in this study between a little bit of play (on average ca. 1 h per week) for the "large-kin-cluster" immatures and virtually nothing (a few minutes per week) for the "small-kin cluster" immatures, could affect the orangutan immatures' (social) development and, therefore, be biologically meaningful.

Assuming that social play is important for the development of their offspring, why would mothers not tolerate play among non-relatives, or only when the other mother is absent? When an independent immature "visits" a mother–offspring pair, the mother, being larger, can easily intervene in the social interaction whenever her offspring signals distress or is risking injury by falling out of the trees. However, if another adult female is also present, she may be less able to quickly rescue her offspring. This might explain why females tend to tolerate the offspring's interactions with well-known maternal relatives, who benefit to some extant from the well-being of both immatures, but are wary or antagonistic towards unrelated females with whom they do not share a common interest. Another explanation may be that it is generally costly for females to spend time in association (potential scramble competition) and when they do this for social reasons (or their offspring's social benefit) prefer a familiar association partner. Familiar partners are likely to impose the lowest physiological cost (lowest stress response) by being predictable and having some shared interest.

Among East African chimpanzee females, who can be almost as non-gregarious as orangutans, severe female aggression has been documented, resulting in the death of a competitor's offspring (Goodall 1986; Pusey et al. 1997; Townsend et al. 2007). Even though orangutans have never been seen to form coalitions in the wild, as in the documented cases of female infanticide in chimpanzees, females have been seen to fight (including biting) with each other on rare occasions. Thus the potential for serious harm to unprotected young immatures is clearly present. In general, mistrust between unrelated females is expected because of the potential harm competing females could do to each other's offspring (cf. offspring-defense hypothesis: Wolff

and Peterson 1998), whereas tolerance is found to be higher among related neighbors (Waser and Jones 1983; Kitchen et al. 2005). It is noteworthy that orangutan females without offspring (nulliparous and females with only older weaned offspring) were more likely to range over a larger area, but retreated into their established range after the birth of their next offspring (van Woerden and Pettersson 2007, and unpublished data). A likely benefit of the more restricted ranging of mothers with young offspring may be that within their smaller range the risk of encounters with strangers is smaller. We have shown here that females respond very differently to encounters with maternally related females *vs*. maternally not related females. This suggest a potential risk posed by unrelated females, even though this may be hard to document.

Dispersal and female relationships in the great ape lineage

This study showed that females of the least gregarious extant great ape actually do maintain social relationships with their philopatric maternal female kin and so seem to enable the development of social bonds among their offspring. In this respect, orangutans are very similar to many other primates living in matrilineal groups, but different from the African great apes which have a tendency for female dispersal with limited opportunity for female bonding. Thus, these results illustrate the variability in philopatric tendencies among all great apes from strongly male-biased to strongly female-biased dispersal.

Perhaps more importantly, the results underscore the fact that orangutan females, in spite of their philopatric tendency, may occasionally live without any nearby adult female relatives. However, the long-term fitness consequences for their offspring remain to be examined. Among the other great apes, females seem to be even more flexible. For example, although chimpanzee females are sometimes able to stay in their natal community and then maintain matrilineal bonds (Goodall 1986; Pusey et al. 1997), they can also form supportive dyadic relationships with particular unrelated females after dispersal (Wakefield 2008; Langergraber et al. 2009; Wild 2010). Bonobo females also form close bonds with unrelated females (Hashimoto et al. 1996) and gorilla females are sometimes able to maintain social bonds with relatives as well as with unrelated females (Watts 1994; Bradley et al. 2007). This ability to form and maintain bonds has freed females from the necessity to be strictly philopatric.

Similar flexibility and independence from philopatry for the formation of intrasexual bonds is also seen among human females. Hill et al. (2011) recently showed that among hunter-gatherers, an individual's or even a female–male pair's site of residence may vary throughout the lifetime. This unusually flexible pattern of dispersal enables a young mother to live near maternal kin and receive their support when she most needs it, but also enables males to co-reside with male kin and form coalitions at other times in their life. As data on great apes accumulate, a similar flexibility in philopatry and dispersal is becoming apparent.

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